Imaging inflammatory lesions by radiolabelled peptides and antibodies

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Introduction

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SPECT radiopharmaceuticals for imaging chronic inflammatory diseases in the last decade. **Anzola LK**, Galli F, Dierckx RA.*Q J Nucl Med Mol Imaging. 2015;59(2):197-213.*

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The role of radiolabelled anti-TNFa monoclonal antibodies for diagnostic purposes and therapy evaluation.
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IMAGING INFLAMMATORY LESIONS BY RADIOLABELLED PEPTIDE AND ANTIBODIES

Inflammation is the physiological response to an invasion by an infectious agent, a foreign antigen, or a tissue lesion, and may entail an acute or chronic process. Acute and chronic forms of inflammation are characterized by a cluster of immunological and histopathological events, which may occur years before the appearance of specific symptoms and can last for years after the clinical diagnosis and the onset of treatment. The inflammatory response to these events usually goes through six different phases that are not necessarily always consecutive in time, and may even coexist in different parts of the affected organs. Imaging procedures like computed tomography (CT) and ultrasound (US) have high sensitivity but lack in specificity for the diagnosis in these conditions. Nuclear medicine techniques, on the other hand, allow the in vivo detection of different physiologic and pathologic phenomena and offer, through the use of non-invasive tools, the possibility to detect early pathophysiological changes before anatomical changes take place. Moreover, these methods could evaluate the degree of disease activity and the efficacy of therapy. Inflammation is not synonymous of infection, but it is rather the response of the organism to the pathogen

Acute inflammation is a reaction of tissues to injuries that is independent of the different possible exogenous noxa. The presence of non-self antigens activates mechanisms such as the release of histamine and serotonin, the increase of vascular permeability, the hyper expression of adhesion molecules on endothelial cells, and the secretion of chemotactic factors. All these phenomena induce leukocyte recruiting along the endothelium and migration of these cells through the capillary wall; complement, antibody production and the release of chemical mediators amplify the local response by the continuous recruitment of cells from the peripheral blood. Some imaging targets in this phase are the neutrophils, the pathogen agents and the activated endothelial cells.

Two main types of chronic inflammation can be recognized: primary and secondary. Primary chronic inflammation is an immune response that follows after infection, after generation of autoimmunity and after development of cancer or organ transplantation. In this type little increase vascularity and permeability and little or no neutrophil infiltration can be recognized. It is usually observed in cell-mediated responses against cells of the body that become the target of the immune system. Imaging targets in this phase are T and B lymphocytes, monocyte/macrophages, and apoptotic cells. Secondary chronic inflammation is due to the persistence of the stimulus that caused the acute inflammation, and the infiltrate is predominantly mononuclear, consisting of lymphocytes and monocytes. Imaging targets in this phase are T lymphocytes and monocytes. Examples of this type of inflammation are sarcoidosis, contact dermatitis and chronic infections as tuberculosis (1). Chronic inflammation is characterized from histological perspective by a)-the presence or absence of edema the migration of activated mononuclear cells such as macrophages and T and B lymphocytes; and b)- the production of cytokines.

Cytokines stimulate collagen production and fibroblast proliferation, which leads to chronic fibrosis with the possible loss of an organ´s functional capacity. Inflammatory chronic diseases include different pathologies that demand the clinician´s timely and accurate diagnosis to plan for the most appropriate therapy. Knowledge of the physiopathological processes of chronic inflammatory conditions facilitates the selection of therapeutic alternatives.

Nuclear medicine offers a wide set of radiolabelled molecules, which allow the identification of the inflammation processes and some metrics that are involved in such diseases. Thus, cytokines, peptides and inflammatory cells can be detected at the inflammation site through the use of radiolabelled monoclonal antibodies and receptor ligands. Molecular nuclear medicine allows the in vivo the characterization of cells, molecules, and phenomena

involved, thus helping to design a tailored personalized treatment. In some cases, it also allows the evaluation of multiple lesions in the same patient that can be asynchronous in presentation and exhibit different histopathological features, requiring different therapeutic approaches. For this purpose, radiopharmaceuticals administered in pico or nanomolar amounts take part in biochemical and physiological processes that allow the visual assessment and quantitation of the inflammatory burden in vivo. Today, it is possible to radiolabel different molecules for use with PET or SPECT systems. PET radiopharmaceuticals systems allow the quantitation of partial volume effect in tissue lesions which is a critical factor during the follow-up of different pathologic conditions. Radiopharmaceuticals for SPECT purposes are labelled with gamma-emitting radionuclides, with lower energy than PET isotopes and a half-life in the order of several hours. Some of the most frequently used are: ^{99m}Technetium, ^{123}Iodine, and ^{111}Indium, with half-lives of 6 hours, 13.1 hours and 2.8 days, respectively.

Scintigraphic images provide important information not only in the diagnostic phase, but perhaps more importantly for therapy decision-making, allowing a selection of the most appropriate therapeutic option for a specific patient and an objective assessment of treatment response. Recent advances in understanding of the molecular basis of chronic inflammatory diseases, the development of target-specific imaging agents, and new advances in the field of medical imaging, allow us to perform non-invasive evaluation of various molecular events such angiogenesis, apoptosis, and cell trafficking in living organisms. Cellular and molecular changes occur long time before structural changes, therefore, non-invasive visualization and quantification of molecular processes facilitate the early detection of disease, establish a prognosis and estimate the potential impact of biologic therapies. Molecular imaging enables a fundamental understanding of key processes for an earlier and more reliable prognosis and assessment of treatment response (2-4). Previous studies with radiolabelled non-specific probes were focused on the disease detection and measurement of the degree of activity.

Nevertheless, no real clinical advantage has been proven when comparing these radiopharmaceuticals to other available diagnostic techniques with regards to patient management. Therapeutic decision is generally based on pathophysiological and patho-biochemical examinations; however, in particular biological therapy can often fail due to the absence or low expression of target molecules in the inflammatory lesions. Therefore, before initiating the treatment, the clinician should ideally know whether the inflammation is active, where it is located, what type of cells are involved and most importantly, what type of receptors or markers are present in the inflammation site.

1. RADIOLABELLED MONOCLONAL ANTIBODIES

Radiolabelled monoclonal antibodies (mAbs) and their fragments against leukocyte antigens can detect infection at an early stage of disease that might be difficult to assess by means of radiological imaging and have several advantages as compared to radiolabelled autologous leukocytes, like easier usage and the target binding with higher specificity. Scintigraphic studies with can detect a specific target molecule among cell populations and are always injected in tracer dose, which rarely induces any clinical side effect in patients. The only adverse reactions described in some cases of murine mAb administration were attributed to the induction of human anti-murine antibodies (HAMA), a host response to foreign antigens. If mAbs are injected in patients with HAMA, these may affect mAbs biodistribution, degrading image quality and affecting the clinical relevance of the study.

Scintigraphy with radiolabelled antibodies can also be used to image inflammatory processes and may offer three exciting possibilities:

1. Better staging of the disease by diagnosing the status of activity in inflammed organs or tissues.
2. Evaluation of therapeutic effect by assessing the activity state before and after treatment.

3. Evidence-based biological therapy by assessing whether a mAb will localize in an inflammed area, such as a joint, before using the same unlabeled mAb as a therapeutic agent.

Several mAbs and their fragments including anti-TNF-α, anti-CD25, anti-CD20, anti-DR, anti-CD3, anti-CD4, anti-MIF, anti-granulocyte and anti-E-selectin antibodies have been labelled with different radionuclides for infection and/or inflammation imaging. Radiolabelled peptides and monoclonal antibodies are highly specific, and a positive scan reveals the presence of the target molecules in the inflammatory lesion. Following this approach, absence of specific targets may provide an explanation for the failure of certain biological therapies (5). The real breakthrough with immuno-scintigraphy for patients with inflammatory diseases is the possibility to identify the presence of the relevant targets involved in the patho-physiology of the disease by means of specific mAbs that will be eventually used, unlabelled, for treatment. Some radiolabelled mAbs (such as anti E-selectin and anti-CD4) demonstrated excellent capacity to visualize inflammation areas but are scarcely utilized for therapeutic purposes, thus limiting further development and use for immuno-scintigraphy. Approval of mAbs for therapeutic purposes has provided an opportunity to select patients through the application of this technique (6). Although radiolabelled mAbs are an interesting class of radiopharmaceuticals, they show some disadvantages that are mainly due to their high molecular weight (7). A Radiopharmaceutical with low molecular weight may show non-specific accumulation in inflammed sites just because of passive diffusion into perivascular space in tissues with increased blood flow, capillary exudate and edema. On the other hand, if the molecular weight is high, sequestration by reticuloendothelial cells may occur and small amounts of mAb remain available for target binding. Both cases will lead to low specificity and diagnostic accuracy. The diagnostic use of radiolabelled antibody fragments with molecular weight of 40-80 kDa could overcome this limitation.

Radiolabelled monoclonal antibodies are identified by a “mAb” suffix because of their animal origin. The radiolabelled mAbs and their Fab fragments were

developed as a new class of radiopharmaceuticals for radioimmunoscintigraphy, which permits imaging of target molecules involved in different immune-mediated disorders (5). These antibodies can be labelled by using different methods with a variety of nuclides, depending on the specific desired diagnostic application and are useful for therapy decision-making. An interesting field of application is in rheumatoid arthritis, where this imaging modality allows better diagnosis and staging of the disease by early detection of inflammed joints, difficult to assess clinically. It also provides the possibility to perform evidence-based biological therapy and to evaluate whether an antibody will localize in an inflammed joint before using the same unlabelled antibody therapy (6).

Anti−TNFα Monoclonal Antibodies

TNFα is potent pro-inflammatory cytokine produced by many cell types, including macrophages, monocytes, lymphocytes, keratinocytes and fibroblasts, in response to inflammation. TNFα exerts its inflammatory effects, both directly on multiple tissue targets and also by inducing other pro-inflammatory cytokines such as IL-1 and IL-6, and acute phase reactants (7). It activates the function of eosinophils and neutrophils and enhances the migration of leukocytes by increasing he permeability of the endothelial layer along with the expression of adhesion molecules in different tissues (8). The primary role of TNFα is the regulation of immune cells, since it exhibits both growth-stimulating and growth-inhibitory properties, and it appears to have self-regulatory properties as well. Deregulation of TNFα production can lead to many systemic complications of infections, such as tuberculosis, and has been implicated in a variety of human diseases (9). TNFα is primary produced as a type II non-glycosylated trans-membrane protein. This newly produced TNFα is inserted into the cell membrane and can be released through proteolytic cleavage of its membrane-integrated form by the metalloprotease TNFα converting enzyme (TACE) to form the soluble trimeric TNFα (10). There are two receptors for TNFα with different molecular sizes: TNF-receptor type 1

(TNF-R1 or p55) and TNF-receptor type2 (TNF-R2 or p75).TNF-R1 is expressed in most tissues, can be fully activated by both the membrane-bound and soluble forms of TNFα and appears to have a critical role in triggering host defense and inflammatory response. TNF-R2 is only found in cells of the immune system, responds to the membrane-bound form and is believed to have a primary role in stimulating the proliferation of T lymphocytes and in suppressing the TNFα- mediated inflammatory response. The main physiological functions of TNFα are to stimulate neutrophils and to attract monocytes to the infectious foci and activate cells to eradicate the microorganisms. These effects are achieved by stimulating the vascular endothelial cells and leukocytes. In severe infections, TNFα is produced in high quantities leading to important local and systemic changes. If the stimuli for the production of TNFα are intense, the quantity of produced TNFα is so high that it reaches the circulation and acts as an endocrine hormone in distant places. In this context, TNFα has a number of actions on various organ systems (11). On the hypothalamus, it produces stimulation of the hypothalamic-pituitary-adrenal axis by promoting the release of corticotropin-releasing hormone, suppressing appetite and inducing fever; increased temperature is mediated by a rise in the synthesis of prostaglandins. On the liver, it produces stimulation of the acute phase response, leading to an increase in C-reactive protein, fibrinoid protein A, and a number of other mediators. When the quantities of TNFα are extremely high, myocardial contractibility and vascular tone may be inhibited, leading to a fall in blood pressure and eventually shock. TNF- α may induce intravascular thrombosis, mainly because of a decrease of the anticoagulant properties of the endothelium. High concentrations of TNFα increase insulin resistance, leading to metabolic changes like a decrease in blood glucose levels. A local increase in TNFα concentrations will cause the cardinal signs of inflammation to occur: heat, swelling, redness and pain, due to up-regulation of adhesion molecules, extravasation of leukocytes, vasodilatation, etc. While high concentrations of

TNFα induce shock-like symptoms, the prolonged exposure to low concentrations of TNFα can result in cachexia, a wasting syndrome.

Several mAbs against TNFα are currently used in inflammatory diseases, of which infliximab and adalimumab are the most widely used. Infliximab is a chimeric IgG 1 mAb with constant sequences (Fc) of human IgG1 (75%) and with murine variable (Fv) domain of mouse origin (25%). It is produced by recombinant cell culture techniques and binds specifically with both soluble and membrane-bound TNFα with high avidity and affinity, forming stable non-dissociating immune complexes (12). It neutralizes the biological activity of TNFα preventing the binding to its receptor. Infliximab inhibits the induction of pro-inflammatory cytokines (interleukin-1 and interleukin-6) and acute phase reactants, the activation of eosinophils and neutrophils, and the migration of leucocytes by downregulating the permeability of the endothelial layer along with the expression of adhesion molecules in synovitis. Studies have demonstrated that infliximab induces cell lysis in transmembrane TNFα expressing cells in-vitro and in-vivo (8,9), mediated by antibody-dependent cellular cytotoxicity (ADCC) or complement dependent cytotoxicity (CDA). Infliximab was approved by the Food and Drug Administration in 1998 for the treatment of moderate to severe active Rheumatoid Arthritis (AR) and posteriorly for sarcoidosis, psoriasis and Behcet´s disease. A disadvantage of Infliximab is that being a chimeric mAb, it may trigger the human anti-chimeric antibody response, with possible reduction of the therapeutic benefit. Adalimumab is the first fully human mAb against TNFα; it is a recombinant human monoclonal IgG1 antibody composed of two kappa light chains (24 kDa each) and two IgG heavy chains (49kDa each), with less immunogenic properties than chimeric mAbs, like infliximab. It has minimal potential side effects and was approved in 2002 for the management of moderate to severe active RA and psoriatic arthritis (13). Adalimumab treatment exerts downregulation of expression of other pro-inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-8 (IL-8), and granulocyte-macrophage colony-stimulating factor (GM-CSF).

Different inflammatory diseases in which TNFα plays a role in their development have been described

Rheumatoid Arthritis

In the development of RA, cytokines generated within the joint tissues stimulate the whole process, with pro-inflammatory cytokines overpowering anti-inflammatory cytokines. Cytokines are believed to play multiple roles in the pathogenesis of RA and include IL-1,IL-2,IL-6,IL-8, TNFα and GM-CSF. TNFα is thought to play a dominant role in the cytokine cascade; it regulates the production of several other pro-inflammatory molecules, stimulates synovial fibroblast cells to express adhesion molecules that attract leukocytes, stimulates neovascularization, activates chondrocytes and osteoclasts to produce proteases with resultant loss of cartilage and bone matrix, and induces fever and other constitutional symptoms.

Ankylosing spondylitis

A major role for pro-inflammatory cytokines has been described in the pathogenesis of this disease. In fact, immunohistochemical analysis of sacroiliac joints in AS patients could detect TNFα as an important cytokine-mediating inflammation factor (14).

Inflammatory bowel disease

Increased levels of production and release of soluble and membrane-bound TNFα in the bowel mucosa of patients with active CD have been found (15). The effects of TNFα in the intestine include disruption of the epithelial barrier, induction of apoptosis of villous epithelial cells, and secretion of chemokines form intestinal epithelial cells (16).

Psoriasis

TNFα not only produced by activated lymphocytes, but also by keratinocytes, dermal chondrocytes, macrophages and mast cells has been demonstrated to play a role in the development of psoriasis (17).

Sarcoidosis

The typical granulomatous inflammation in this disease is primarily characterized by accumulation of monocytes, macrophages and activated T-lymphocytes, with an increased production of key inflammatory mediators such as TNFα, INFγ, IL-1, IL-2 and IL-12. TNFα is involved in granuloma formation and subsequent fibrogenesis, acting synergistically with IL-1 which is released locally at increased levels due to the stimulatory activity of TNFα (18).

Scintigraphic possibilities of labelled anti- TNFα mAb for diagnosis and therapy evaluation

Infliximab was radiolabelled with ^{99m}Tc using a direct radiolabelling method after disulphide-bridge reduction with 2-mercaptoethanol as described by Mather et al., with labelling efficiency of more than 97% (19). Nevertheless, being a chimeric mAb, infliximab may trigger the human anti-chimeric antibody response, possibly affecting the therapeutic benefit and efficacy, and potentially inducing false negative results in scintigraphy.

Rheumatoid Arthritis

The first clinical study with labelled infliximab in RA patients was performed by Conti et al. (2005) who demonstrated high levels of TNFα using anti-TNFα scintigraphy in a patient with refractory monoarthritis of the knee, successfully treated with intra-articular infliximab. The impressive and sustained clinical response demonstrated the potential role of TNFα in the pathogenesis of this kind of monoarthritis. The clinical and laboratory improvement was associated with negativization of scintigraphic findings, which showed absence of detectable levels of TNFα in the affected knee. The authors suggested that the

selection of patients who are candidates for this innovative intra-articular therapy should be guided by anti-TNFα scintigraphy (20). Barrera et al. obtained similar results after studying 10 RA patients with ^{99m}Tc-infliximab scintigraphy, reporting high specificity in imaging of inflamed joints(21). In a study involving 12 cases, Signore et al. demonstrated that ^{99m}Tc-infliximab scintigraphy could be a useful tool to predict clinical response to intraarticular infliximab in patients with refractory monoarthritis (22). In a pilot study with 9 patients who received intra-articular infliximab, Chianelli et al., showed significant decreased uptake in three joints, and slightly reduced uptake in 4 joints after the procedure by using radiolabelled infliximab pre and post therapy. Their results provided relevant information about the potential usefulness of this molecule for evaluating residual presence of TNFα in joints and providing a rationale for an eventual new cycle of therapy (23). Malviya et al. demonstrated promising results for therapy decision-making and follow-up using ^{99m}Tc-anti-TNFα and ^{99m}Tc-anti-CD20 in patients with RA, assessing whether an antibody will accumulate in an inflammed joint before using the same unlabelled antibody for therapeutic purposes (24). Their results showed that both ^{99m}Tc-labelled antibodies accumulated significantly in the majority of painful joints, but not in all joints, and not in a similar fashion. For each antibody, the uptake in inflammed joints seemed to correlate with the presence of its target and the activity status of the disease, thus predicting the efficacy of therapy performed with the same cold antibody. Another group used anti-human TNFα labelled with ^{99m}Tc to evaluate joint inflammation in eight RA patients. The sensitivity and specificity for the nuclear imaging technique versus MRI were 89.9% and 97.3% respectively (25). From studies in RA patients emerges that imaging modalities with ^{99m}Tc-infliximab allow the evaluation of TNFα uptake in active inflamed joints, and this finding seems to predict the efficacy of therapy with the same agent.

Crohn's Disease

D'Alessandria et al. performed a study in patients with Crohn´s disease using ^{99m}Tc-infliximab evaluating the in- vivo biokinetics of the TNFα antibody and its ability to predict the clinical response to therapy. They also reported the results of a comparison between ^{99m}Tc-infliximab and ^{99m}Tc-leukocyte scans; the ^{99m}Tc-infliximab study showed the presence of little TNFα in the affected bowel of patients with Crohn´s disease (26).

Complex Regional Pain Syndrome

Bernateck et al. reported the findings on 3 patients with complex reflex painful syndrome (CRPS) of the hand, showing that radiolabelled anti-TNFα localized only in affected hands of patients with type 1 CRPS, with no uptake in clinically unaffected hands. In late-stage CRPS, findings supported the evidence for neuroimmune disturbance in patients with CRPS, which may have further implications for specific anti-cytokine treatment (27).

Similarly to infliximab, adalimumab has been labelled with ^{99m}Tc via an indirect method described by Abrams et al., with an efficiency greater than 95% (28). Barrera et al. reported a study in 10 patients with active RA to assess the sensitivity and biodistribution of i.v. administered ^{99m}Tc-adalimumab (21). Each patient underwent 2 scintigraphic examinations: the first one, to evaluate the biodistribution of the radiolabelled antibody, and the second one after 2 weeks, to assess the specificity for TNFα targeting and the sensitivity for changes in degree of inflammation. The results of the first scan showed that inflammed joints were clearly visualized at 4 and 24 hours after the injection, with a median increase of about 30%. Not all clinically expressive joints showed uptake of ^{99m}Tc-adalimumab, which is probably explained by the absence of this cytokine in some inflammed joints. For the second scan, the group was divided in two: one group received a therapeutic dose of unlabelled adalimumab, and the other an intramuscular corticosteroid, just before the injection of ^{99m}Tc-adalimumab. The results showed a reduction of 25% of the joint uptake in the group that had received adalimumab. In the second group,

there was a decrease in the uptake of the radiopharmaceutical, suggesting the usefulness of the ^{99m}Tc-adalimumab for the detection of clinically relevant changes in disease activity.

^{99m}Tc-Infliximab vs ^{99m}Tc-Adalimumab

In a study reported by Annovazzi et al. (29) ^{99m}Tc-infliximab and ^{99m}Tc-adalimumab were used for therapy decision-making and follow-up in RA patients. No biodistribution differences were found between the two radiopharmaceuticals and a variable degree of joint uptake was observed, which was not always correlated with the presence of joint pain or swelling. They also observed that both radiotracers showed high affinity for inflammation areas, with no uptake in normal tissues and no human antibody response. After therapy with unlabelled anti-TNFα, a decrease in joint uptake of ^{99m}Tc-anti-TNFα was correlated with a reduction of sicca symptoms. Additionally, they observed that patients who showed high uptake in pre-therapy scans had more therapeutic benefits than those who showed lower uptake in the inflammed joints. They concluded that ^{99m}Tc-TNFα antibodies could be used for decision-making in patients with active RA, predicting the successof therapy with the same unlabeled mAb. Malviya et al. (24) used radiolabelled infliximab and adalimumab for therapy decision making and follow-up of patients with RA. They also observed more therapeutic benefit inpatients that showed high pretreatment uptake compared to those who showed lower uptake in the inflammed joints before treatment. Scintigraphic scores were found to be reliable for disease monitoring and therapy decision-making.

In summary, scintigraphic imaging of TNFα may be of importance in the following situations:

- detection of cell-bound and soluble TNFα to localize sites of inflammation in patients suspected of inflammatory disease;
- demonstration of inflammation sites in cases suspected of inflammation but without a systemic inflammatory response;

- prediction of therapeutic response to treatment with TNFα blocking agents.

The available evidence is highly encouraging and promising for therapy decision-making and follow-up, with a view on assessing whether and antibody will accumulate in an inflammed joint before using the same unlabelled antibody for therapeutic purposes. This methodology is known as the “pre-therapy receptor mapping approach” for therapy decision-making, and it may also provide a cost-effective solution for highly expensive biological therapies.

Anti-CD20 mAbs

Rheumatoid arthritis, psoriatic arthritis, Sjögren’s syndrome, dermatopolymiositis, sarcoidosis, Behcet´s disease are chronic inflammatory autoimmune diseases and treatment is often complicated. It has been shown that targeting B cells can directly alter autoimmune responses in patients with these disorders (30). B lymphocytes are responsible for the production of auto-antibodies and rheumatoid factors, are also involved in T lymphocyte activation and pro-inflammatory cytokine production, and play an important role in the pathogenesis of inflammatory autoimmune diseases (31). Such cells have been found in pathological infiltrates in affected tissues of patients with autoimmune diseases and are implicated in disease progression (32). The development of mature B lymphocytes from stem cells involves several stages, each one of which modifies the expression of a wide range of surface markers. Thus, there are several potential scenarios in which B lymphocytes depleting therapies can act directly via use of mAbs against cell surface markers (such as CD19, CD20, CD22) or indirectly via blockage of cytokines pathways (such as TNFα, interleukin-6, B lymphocyte stimulator (BLyS) and proliferation-inducing ligand (APRIL), (33, 34). CD20 is expressed in over 95% of B lymphocytes from blood and lymphoid organs, which makes it a suitable target for immunotherapy purposes (35).

CD20 is an unglycosylated transmembrane protein that forms part of a multimeric cell-surface complex that regulates calcium transport involved in the moderation of B-lymphocyte activation and proliferation (36). In the last years, biological therapies for the treatment of rheumatologic and autoimmune disease are rapidly expanding, and B lymphocytes represent a main target for this new approach. Currently, the most widely used mAb for treatment of rheumatological diseases is rituximab, an IgG1k subclass chimeric murine/human anti-CD20 antibody (37). Anti-CD20 antibodies cause the death of target cells through several mechanisms:

1- activation of the complement cascade.

2- killing of antibody-coated cells by cells expressing Fc receptors.

3- direct anti-proliferative effect or apoptosis (38).

Anti-CD20 therapies include the humanized anti-CD20 mAbs ocrelizumab, veltuzumab and ofatumumab, that vary in the extent of humanization. They also have different complement-dependent cytotoxicity and antibody-dependent cell-mediated cellular cytotoxicity (39). As it has been already mentioned, rituximab is an IgG1k isotype chimeric anti-CD20 mAb that binds specifically to the transmembrane CD20 antigen. It was the first chimeric mAb approved by the FDA for the treatment of lymphoma and for patients with active arthritis that do not respond to one or more necrosis factor antagonist therapies. This new generation of mAbs can also be radiolabelled by using a direct or indirect method with a variety of nuclides depending on the specific diagnostic application. For RA patients, several mAbs and their fragments, including anti TNFα, anti-CD20, anti-CD3, anti-CD4 and anti-E-selectin antibody have been radiolabelled, mainly with ^{99m}Tc or ^{111}In. Scintigraphic studies with these antibodies offer an exciting possibility to study RA patients, providing two types of information:

a) they allow better staging of the disease and diagnosis of the state of activity by early detection of inflammed joints that might be difficult to assess.

b) they might aid in performing “evidence-based biological therapy” of arthritis assessing whether an antibody will localize in an inflammed joint before using the same unlabelled antibody for therapy.

This might be particularly important for the selection of patients to be treated since biological therapies can be associated with severe side-effects and are considerably expensive. Malviya et al. (6) demonstrated that rituximab can be radiolabelled with high efficiency without affecting its biological activity and specificity for CD20 receptors in vivo. They observed different biodistribution of radiolabelled CD20 in different patients, reflecting different degrees of B lymphocyte infiltration in tissues across patients. The results hold promise not only for localizing lymphocyte infiltration in inflammatory diseases, but also for mapping B lymphocytes in affected tissues of each patient in order to establish a personalized therapy. In another study (40), the same authors reported that after the administration of ^{99m}Tc-rituximab it was possible to detect accumulation in the inflammed regions in coincidence with clinical data. They also compared two tracers and reported different degree of uptake in different joints (^{99m}Tc-anti-CD20 and TNFα), assuming selective inflammatory pathways in different joints that might benefit from targeted therapies. No allergic reactions or side-effects were reported.

Anti-CD4 mAbs

CD4 is a kDa monomer membrane glycoprotein expressed on T-lineage cells. The extra-cellular domain of CD4 binds to the conserved regions of MHC II molecules on antigen-presenting cells. CD4+ T lymphocytes constitute the helper subset that regulates T and B lymphocyte function during T lymphocyte- dependent responses. CD4 cells and their cytokine products play an important role in RA and several autoimmune diseases (41). Indeed, a number of anti-CD4 mAbs are available for treatment of different autoimmune disorders (42). Several studies using radiolabelled anti-CD4 mAbs have been published demonstrating high specificity for

inflammation in autoimmune diseases. Becker et al. (43) by using anti-CD4 labelled with ^{99m}Tc demonstrated in 6 RA patients how this molecule could specifically detect CD4+ cells in affected tissues. Kinne et al. (44) used radiolabeled anti-CD4 antibodies for imaging inflammed joints in 8 patients with RA, and showed how anti-CD4 mAb allowed highly specific detection of inflammatory infiltrates rich in CD4-positive cells. The authors also proposed that this radiotracer could be used to differentiate chronic joint inflammation versus septic processes, because of the ability to bind to mononuclear cells chronically infiltrating inflammed joints. The same authors (45) demonstrated how anti-CD4 imaging was superior to other methods, and concluded that since CD4 antigen is present on the surface of T-helper lymphocytes and macrophages infiltrating the synovial membrane, imaging with anti-CD4 may allow more specific detection of inflammatory infiltrates in RA.

Anti-CD3 mAbs

A humanized non-FcR binding derivate of the anti-human CD3 monoclonal antibody, OKT3, can induce generalized immunosuppression in patients with psoriatic arthritis (46, 47). This antibody could cause modulation of the CD3 T lymphocyte receptor complex, induction of clonal energy and/or induction of regulatory T lymphocytes production. An anti-CD3 IgG2 murine mAb, muromonab, has been directly labelled with ^{99m}Tc and Marcus et al. (48) in 7 RA and 2 psoriatic arthritis patients showed a positive scan in 8 out of 9 patients, even when potent immunosuppressive and anti-inflammatory drugs had induced good clinical response to pain. They observed that all 34 studied joints with moderate-to-severe pain had moderate to marked radiopharmaceutical uptake. Two patients experienced side effects, leading to a conclusion that ^{99m}Tc-anti-CD3 mAb could be useful in measuring therapeutic effectiveness in RA patients, but with side effects profile limiting the potential use in clinical imaging. Martins et al. (49) reported the feasibility of using the mAb (OKT3) labelled with

^{99m}Tc to monitor disease activity in 38 patients with diagnosed RA. The scintigraphic findings showed significant correlation between the accumulation of ^{99m}Tc-OKT3 and swollen and tender joints, and the visual analogue scale. They were able to differentiate patients in remission from those with active synovitis, according to DAS 28. In contrast, there was no correlation between uptake and the patient´s age, gender, duration of disease, or erythrocyte sedimentation rate. They concluded that ^{99m}Tc-OKT3 scintigraphy is a reliable and objective method for detecting synovial activity and can be used to observe disease prognosis. Malviya et al. (50) reported their experience radiolabelling visilizumab with ^{99m}Tc, and they demonstrated encouraging results for the targeting of CD3+ human lymphocytes in an in-vitro and in-vivo animal model.

2. RADIOLABELLED PEPTIDES

The majority of peptides used in nuclear medicine are constituted by a relative small number of amino-acids (up to 30) that do not show a well-defined tertiary structure. In contrast to bigger proteins and antibodies, peptides can be easily synthesized, stabilized and modified to obtain good pharmacokinetic parameters. Major features of peptides are: a) rapid pharmacokinetics; b) modifiable excretion route; c) can be biologically active and usually not immunogenic (51). Compared to larger molecules, small peptides are rapidly taken up and retained in target tissues, in accordance with the usually rapid plasma clearance due to fast renal excretion. Also, they generally show high receptor binding affinity and are internalized into the cells. Radiolabelling of peptides has been performed using a bifunctional chelating agent.

As far as radiolabelled peptides is concerned, it is important to emphasize the increasing use of somatostatin (SST) analogues targeting somatostatin receptors (SSTR) in inflammatory diseases, particularly in RA, Sjrögen Syndrome (SS) and autoimmune thyroid diseases. High diagnostic accuracy is obtained due to the high binding affinity of SST to its five

receptors. Different SST analogues have been proposed in clinical practice; among all synthetic compounds, Pentetreotide (Octreotide®) labelled with ^{111}In has been the most widely used for imaging purposes, due to its high affinity for SSTR 2 and 5. New radiopharmaceuticals for somatostatin receptor scintigraphy (SRS) showing a different and/or wider affinity to receptors are now available and labelling has been obtained both with gamma and positron emitters for diagnostic purposes using SPECT or PET, respectively (52). The labelling of octreotide and other analogues has been carried out by conjugation of the peptide with a bi-functional chelator such as DTPA, DOTA or NOTA (53), and subsequent addition of a radionuclide like ^{123}I, ^{111}In, ^{99m}Tc, ^{68}Ga, ^{18}F and ^{64}Cu (54).

SST is a cyclic hormone that regulates several physiologic cell processes via specific receptors (SSTR) that are expressed by nerve cells, many neuroendocrine cells and inflammatory cells such as lymphocytes, monocytes, monocytes/macrophages, peripheral blood mononuclear cells and thymocytes (55). Five SSTR subtypes have been cloned which have been found in cells involved in inflammatory responses with high density expression observed in neoangiogenic and peritumoral vessels, epithelioid cells, proliferating synovial vessels and activated lymphocytes and monocytes (56). For diagnostic purposes, radiolabelled SST analogues have been used in SPECT and PET with clinically relevant results in different chronic inflammatory diseases, particularly for the evaluation of disease activity, prognosis and therapy follow-up (57, 58). The role of somatostatin as a mediator in inflammatory processes and the way different immunological cells express SSTRs is well known. The possibility to specifically target SSTRs by molecular imaging has given the opportunity to visualize active inflammation in a variety of disorders.

Endothelial inflammation and atherosclerosis

It is well known that monocytes and macrophages, being the first cell lines associated with vascular inflammation, play an important role in the

pathogenesis of atherosclerosis, contributing to necrotic core formation, fibrous cap thinning, and plaque vulnerability (59). These cells express SSTR-2 on the surface in cultures (60) and therefore ^{68}Ga-DOTA-TATE was thought to have the potential to be a surrogate marker of inflammation when studying plaque biology (61). In a systematic review conducted by our group, we found 7 studies with a total of 262 patients aiming to investigate the utility of radiolabeled somatostatin receptors in endothelial inflammation (62-68). The analysis of pooled data strongly suggests the existence of complex relationships between plaque inflammatory stage (chronic vs acute inflammation), macrophage density and activity, SSTR subtype expression, and ^{18}F-FDG and ^{68}Ga-DOTA-TATE uptake. So far, available evidence tends to confirm the utility of SRS with ^{68}Ga-DOTA-TOC to detect high risk, vulnerable atherosclerotic plaques and an interesting correlation with risk factors. Moreover, it appears to exist an added value of DOTA-TOC (specific for inflammatory cells only provides an in-vivo information on the vulnerability of plaques) over FDG (global inflammatory burden but nonspecific, not providing relevant histopathological information), as well as ^{18}F-Na calcium score (69).

Rheumatoid Arthritis

Synovia of patients with active rheumatoid arthritis express high density SSTR (70). The experience published to date (71,72) shows the potential of SRS to localize and identify sites of active inflammation in joints, both symptomatic and asymptomatic, as well as extra-articular involvement like in salivary glands. The information provided by SRS differs from other imaging modalities and provides in-vivo histopathological insight on the activity of cell-mediated inflammation. This information has an important impact for therapy decision making and therapy follow-up, as reported in other studies (73).

Idiopathic pulmonary fibrosis

Among the different available tracers, ^{68}Ga-DOTA-NOC presents the broader SSTR-subtype affinity (74), a favorable dosimetry, and no uptake in the intact lung (75). Recent preclinical evidence demonstrated SSTR expression on fibroblasts of both murine models of Idiopathic pulmonary fibrosis (IPF) and human tissue samples from IPF patients (76). Although ^{68}Ga-DOTA-NOC binding sites within the lungs cannot be precisely localized without direct sampling, the fact that tracer uptake was observed in IPF cases allows to speculate that PET imaging with ^{68}Ga-DOTA-NOC might be useful to identify SSTR overexpression in this patient subgroup. ^{68}Ga-DOTA-NOC PET/CT can be used for IPF patients, selecting those who might benefit from somatostatin analog treatment or combined approaches in which ^{68}Ga-DOTA-NOC may function as a carrier for specific anti-fibrotic drugs or cytotoxic isotopes. We remark the experience reported by some authors (77-79) showing the potential utility of SSTR scintigraphy to detect active inflammation mediated by an immunological response in IPF.

Graves' ophthalmopathy

Significant results can be extracted from 13 studies with a total of 405 patients. In similar studies using ^{111}In Octreotide Aguirre-Balsalobre et al. (80), Colao et al. (81), Gerdin et al. (82), Kahaly et al. (83, 84), Krassas et al. (85, 86), Moncayo et al. (87), Nocaudie et al. (88), Postema et al. (89), demonstrated increased tracer uptake in the orbits of patients with Graces' disease and active orbitopathy. These authors concluded that this finding was helpful to discriminate active vs non-active disease and helped to better choose the candidates for immunotherapy, radiotherapy or somatostatin-analogues therapy. In those series, it was possible to observe how patients with higher orbital uptake responded well to the installed treatment. Similar results were reported by Burggasser et al. (90) using ^{99m}Tc-P-829 and Huang Sung et al. (91) using ^{99m}Tc-HYNIC-TOC. Lincke

et al. (92) are the only known investigators that used PET in this pathology. They compared the uptake characteristics between ^{111}In-Pentreotide and ^{68}Ga-DOTA-TOC in normal and altered thyroid glands and found that both tracers showed high uptake in active Hashimoto and Graves' disease, most likely caused by SSTR-expressing lymphocytes in thyroid tissue.

During the last 20 years, the utility of SSTRs scintigraphy for identifying active orbitopathy in Graves' exophthalmopathy has been demonstrated. Several authors have consistently reported similar scintigraphic patterns of high uptake of the labeled molecule in the periorbitary zone as evidence of active disease, with prognostic implications.

Sarcoidosis and other chronic inflammatory diseases

The use of ^{111}In-pentetreotide in chronic inflammatory diseases was first reported for the evaluation of granulomatous diseases such as tuberculosis, Wegener granulomatosis and sarcoidosis (93). In vitro autoradiography performed on biopsy specimens of sarcoid tissue indicated that somatostatin receptors (subtype 2) were present in epitheloid cells and giant cells, probably the sites of in vivo ^{111}In-pentetreotide binding. In sarcoidosis patients, uptake of ^{111}In-pentetreotide decreased with successful corticosteroid therapy, suggesting that tracer binding reflected active sites of the disease (94). In an observational study of 15 patients with sarcoidosis and suspicion on cardiac involvement, Lapa et al. (95), assessed the feasibility of SRS with ^{68}Ga-DOTA-TOC by PET/CT for detecting cardiac sarcoidosis in comparison to cardiac MRI (cMRI); overall concordance of the 2 modalities was 96.1%. In a cross-sectional study in 46 patients with known mediastinal, hilar and interstitial sarcoidosis using SRS with ^{111}In-pentetreotide, Kwekkeboom et al. (94) showed positive results in 36 out of 37 patients with known pathology, and in 7 with normal X-rays. In 13 patients with no evidence of extrapulmonary disease, SRS revealed abnormal uptake of the tracer outside the chest. In an

observational study, Lebtahi et al. (96) demonstrated the superiority of ^{111}In-pentetreotide compared with ^{67}Ga-citrate scintigraphy in the evaluation of pulmonary and extrapulmonary involvement in 18 patients with proven sarcoidosis, especially in the hilar or mediastinal areas.. In a retrospective study, Kamphuis et al. (97) evaluated the additive value of SRS scintigraphy with ^{111}In- pentreotide in the clinical evaluation of sarcoidosis and compared the results with chest X- ray and CT. In both histologically proven and unproven sarcoidosis, in all cases but one, SSTR uptake was demonstrated. In the thoracic region, SRS increased the diagnostic yield by more than 30% in comparison with X-ray and CT. In the histologically proven group, there were no negative SRS results, and the SRS increased the yield for thoracic localization in 30% and 14% of the patients in comparison with X-ray and CT, respectively. In a pilot study involving 19 patients with suspected cardiac sarcoidosis, Gormsen et al. (98) compared the diagnostic accuracy and inter-observer variability of ^{68}Ga-DOTA-NOC vs ^{18}F-FDG PET/CT. ^{68}Ga-DOTA-NOC correctly classified all patients, showing excellent diagnostic accuracy.

In a descriptive study, Nobashi et al. (99) compared the utility of ^{68}Ga-DOTA-TOC with conventional ^{67}Ga-citrate for the identification of active sarcoidosis and correlated quantitative parameters on ^{68}Ga-DOTA-TOC PET/CT with clinical data. ^{68}Ga-DOTA-TOC was superior than ^{67}Ga-citrate, for identifying significantly more number of involved lymph nodes ($p<0.046$). An observational study by Piotrowoski et al. (100) in, evaluated 32 patients with sarcoidosis and used ^{99m}Tc-HYNIC-TOC scintigraphy as a reference method to assess the clinical usefulness of traditional markers. They found a significant difference between negative and positive SRS when patients were defined according to level of lipid peroxidation in exhaled breath condensate (EBC 8-IP).

SRS has been also proposed for studying other chronic inflammatory conditions, in particular in histiocytocis, tuberculosis, cardiac allograft rejection and small vessel vasculitis. Weinmann et al. (101) evaluated 13

patients with histiocytosis; lung uptake of ^{111}In-pentreotride by visual and semiquantitative analysis was significantly higher in patients compared to controls. Oztürk et al. (102) described similar results in three cases: 2 patients with pulmonary sarcoidosis and one with tuberculosis studied with ^{111}In-pentreotide scintigraphy. Aparici et al. (103) conducted a prospective study in 13 patients with suspected cardiac allograft rejection to assess the feasibility of SRS with ^{111}In-penteotride to target activated lymphocytes in transplanted hearts as a possible early marker of rejection. High cardiac uptake was observed in 8 patients: 3 had acute rejection and 5 had mild or no rejection. However, in 4 of these 5 patients , a biopsy performed one week later demonstrated significant rejection requiring treatment. These preliminary results indicate the feasibility of targeting activated lymphocytes for the detection of cardiac allograft rejection and suggest a possible predictive role of SRS. Neumann et al (104) analyzed 32 consecutive patients with ANCA-associated small vessel disease (AASV). Disease activity was evaluated with the Birmingham Vasculitis Activity Score (BVAS). For pulmonary AASV, SRS showed a sensitivity of 86% and a specificity of 96%, with a positive predictive value of 97% for active disease. False negative scans were seen in patients under immunosuppressive therapy. In patients with ear/nose/throat involvement, SRS showed a sensitivity of 68% and a specificity of 100%, with a positive predictive value of 100%. In patients who responded to therapy and evolved into full remission, repeat SSTR scintigraphy demonstrated the absence of previously present tracer accumulation. Patients with aggressive disease who responded poorly to immunosuppressive therapy remained positive at repeat scintigraphy. Reported evidence to date shows the utility of ^{68}Ga-DOTA-TOC for detecting sarcoid lesions, especially for lymph nodes, uvea and muscles, as well as for vasculitis and other chronic inflammatory diseases. In particular, the results obtained in monitoring cardiac allografts opened a new important clinical application of SRS to early detect

lymphocytic infiltration in transplanted tissues and to monitor the effect of therapies in preventing rejection.

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Chapter 2

Somatostatin receptor imaging by SPECT and PET in patients with chronic inflammatory disorders: a systematic review.

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Abstract

Objective**:** To review the literature on the clinical application of radiolabeled somatostatin receptor scintigraphy (SRS) by SPECT and PET in adults with chronic inflammatory diseases.

Research design: Systematic review of published observational studies between 1993 and 2017.

Data collection and analysis**:** Cochrane Central Register of Controlled Trials, MedLine, EMBASE, PubMed, Google Scholar, OVID, EBSCO, Scopus and

Web of Science were used to search for studies on the use of SRS in adults with chronic inflammatory diseases. A team of reviewers independently screened for eligible studies. Quality of evidence was assessed by Quadas approach.

Results: Eligible papers included 38 studies. Studied populations were heterogeneous, and patients were classified according to the diagnosed disease: endothelial inflammation, rheumatoid Arthritis, cardiac allograft rejection, granulomatous diseases, small vessel vasculitis, idiopathic pulmonary fibrosis, sarcoidosis and thyroid exophthalmopathy. Because of many quality differences between studies, it was not possible to pool data, and a narrative synthesis is reported.

Conclusion: Results highlight the value of SRS to detect active inflammation in several chronic inflammatory conditions, despite the bias related to the index test, showing lack of standardization of the scintigraphic technique and high variability of methods used to clinically evaluate inflammatory condition.

Key words

Somatostatin receptor imaging- SPECT- PET- Inflammatory diseases.

Introduction

Chronic inflammatory diseases, are characterized by long-standing mononuclear cell infiltration of the target organ, leading to hypofunction and requiring life-long treatment (1). In the past years several different radiopharmaceuticals have been synthetized for molecular nuclear medicine that may contribute to the diagnosis and prognosis of these diseases (2). In particular, several peptides, receptor ligands and monoclonal antibodies have been radiolabeled, allowing the in vivo visualization of the inflammatory process at cellular and molecular level (3).

As far as radiolabeled peptides are concerned, it is important to emphasize the increasing use of somatostatin (SST) analogues targeting somatostatin receptors (SSTR) in inflammatory diseases, particularly in rheumatoid arthritis (RA), Sjögren's syndrome (SS) and autoimmune thyroid diseases. Because the broader spectrum of interaction with SSTR in other different pathological conditions than neuroendocrine tumors, such as chronic inflammation, and because the known presence of SSTR over-expression by inflammatory cells, immunological cells in different tissues, blood vessels among others, it has been possible to use different radiolabelled somatostatin analogues with diverse affinity for these receptors, for diagnostic purposes in different oncological and inflammatory scenarios (4).Different SST analogues have been proposed in clinical practice. Among all synthetic compounds Pentetreotide (Octreotide®), labelled with ^{111}In, was the most widely used for imaging purposes, because of its high affinity for SSTR 2 and 5. New radiopharmaceuticals for somatostatin receptor scintigraphy (SRS), showing a different and/or wider affinity to the receptors, are now available and labeling has been obtained both with gamma and positron emitters for diagnostic purposes, with SPECT or PET, respectively, such as: ^{68}Ga-DOTA-TATE and ^{64}Cu-DOTA-TATE(affinity to type 2 receptors), ^{68}Ga-DOTA-TOC (more selective to 2 and 5 type receptors), ^{68}Ga-DOTA-NOC (affinity to 2,3 and 5 type receptors), ^{99m}Tc-EDDA/HYNIC-TOC(affinity to 2 and 5 type receptors) (5).

The labeling of octreotide and other analogues has been carried out by conjugation of the peptide with a bi-functional chelator, DTPA, DOTA or NOTA (6), and subsequent addition of a radionuclide such as ^{123}I, ^{111}In (pentreotide), ^{99m}Tc (depreotide-EDDA/HYNIC-TOC) or ^{68}Ga, ^{18}F and ^{64}Cu (DOTATATE) (7). SST was first isolated from ovine hypothalamic extracts and was characterized as a tetradecapeptide. It was identified as part of the releasing hormone family for its property to inhibit the secretion of growth hormone from pituitary cells by Brazeau and colleagues in 1973 (8).SST-producing cells are typically neurons or endocrine-like cells and are found in high density in the central and peripheral nervous systems and in the endocrine pancreas and in the gut and in small numbers in the thyroid, adrenals, submandibular glands, kidneys, prostate, placenta blood vessel walls, and immune cells (9). It is a cyclic hormone that regulates several physiological cell processes via specific receptors (SSTR) which are expressed by nerve cells, many neuroendocrine cells and inflammatory cells such as lymphocytes, monocytes, monocytes/macrophages, peripheral blood mononuclear cells and thymocytes (10). Five SSTR subtypes have been cloned which have been found in cells involved in inflammatory responses with high density expression observed on neoangiogenic and peritumoral vessels, epithelioid cells, proliferating synovial vessels and activated lymphocytes and monocytes (11). In the peripheral blood mononuclear cells and in the spleen, mainly SSTR subtypes 2 and 3 are found; in the thymus mainly SSTR subtypes 1, 2, and 3; in macrophages and in dendritic cells mainly SSTR subtype 2; in B lymphocytes mainly SSTR subtype 3; and in T lymphocytes SSTR subtypes 1 to 5 (4). For diagnostic purposes, radiolabelled SST analogues have been used in SPECT and PET with clinically relevant results in different chronic inflammatory diseases, particularly for the evaluation of disease activity, prognosis and therapy follow-up (12,13). Although 18F-fluorodeoxyglucose (18F-FDG) PET/CT has recently gained a role also in infective and inflammatory disease, because logistically is easier and quicker to perform, this radiopharmaceutical is not specific and therefore it is not able to discriminate an infection from an inflammation (14).

In this review we systematically analyze all papers on SRS (with either SPECT or PET) in the setting of various chronic inflammatory diseases: endothelial inflammation, rheumatoid arthritis, cardiac allograft rejection, small vessel vasculitis, idiopathic pulmonary fibrosis, sarcoidosis and granulomatous diseases, and Graves' ophthalmopathy.

Why is important to do this review: it important to do this review because the literature has shown the potential utility of radiolabeled somatostatin analogues not only in the diagnostic setting but as prognostic factor and as part of treatment control in some chronic inflammatory diseases, it is important to assess and collect the best evidence published up today regarding to this topic.

Methods

Criteria for considering studies for this review

Types of studies: Observational cohort studies, systematic reviews, reports of more than three cases.

Types of participants: Adult patients with any diagnosed inflammatory chronic disease such as rheumatoid arthritis, sarcoidosis, cardiac allograft, atherosclerotic plaques, Sjögren's disease, idiopathic pulmonary fibrosis, graves exophthalmopathy who had a radiolabeled somatostatin receptor analogue scintigraphy for diagnostic purpose.

Types of intervention: Somatostatin receptor analogues radiolabeled with Spect tracers:^{111}In Octreotide, ^{99m}Tc:^{99m}Tc-EDDA/HYNIC-TOC, ^{99m}Tc P829 and with PET tracers: ^{68}GaDOTA- NOC, ^{68}Ga DOTA-TOC, ^{68}Ga DOTA-TATE, ^{64}Cu DOTATATE.

Types of study outcome: The final diagnostic for the entire population was related to chronic inflammatory diseases such as: rheumatoid arthritis, Sjögren's disease, Graves exophthalmopathy, atherosclerotic plaques, idiopathic pulmonary fibrosis, sarcoidosis and other inflammatory conditions.

Search method for identifying the eligible studies

The selection of appropriate publications was based on the PRISMA guidelines (15). We searched in Cochrane Central Register of Controlled Trials

(CENTRAL) published in the Cochrane Library, MedLine (1993-2017), EMBASE (1993-2017), PubMed (1993-2017), Google Scholar (1993-2017), OVID (1993-2017), EBSCO (1993-2017), Scopus and Web of Science (1993-2017). All relevant studies published in English, Italian and Spanish language were identified by using the following search strategy: ("receptors, somatostatin"[MeSH Terms] OR "receptors"[All Fields] AND "somatostatin"[All Fields]) OR ("somatostatin receptors"[All Fields] OR ("somatostatin"[All Fields] AND "receptor"[All Fields]) OR "somatostatin receptor"[All Fields]) AND ("Bildgebung"[Journal] OR "imaging"[All Fields]) AND ("adult"[MeSH Terms] OR "adult"[All Fields] OR "adults"[All Fields]) NOT ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "neoplasm"[All Fields]) NOT ("tumours"[All Fields] OR "neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "tumors"[All Fields]) somatostatin receptor imaging AND adults.

Inclusion criteria:

Studies or reports in which compounds were labeled with ^{68}Ga, ^{99m}Tc, ^{68}Ga, ^{64}Cu for applications other than in oncology-related indications were included. Every effort was made to include both the earliest and the most recent publications relating to a particular application, as well as any study with a significant new contribution. The decision to include or exclude an article was made by consensus.

Exclusion criteria:

Publications in which the focus was on SRS in oncology-related applications or in which the emphasis was on aspects related to generators, radiochemistry, animal models, experimental reports or physics, were excluded.

Literature search was broadened to all reference lists of all retrieved articles, including observational studies, systematic reviews, conference proceedings and poster presentations. Studies or reports with more than three cases in which SRS was used in patients with inflammatory diseases were also included. Case reports allusive to just one patient where not taken in consideration because the results of the observations could not be compared between patients in order to highlight differences or similarities.

Selection of studies data extraction and management:

The decision to include or exclude an article was made by consensus between LKF, AWJMG, AS and AM. We used the search strategies described to obtain titles and abstracts of studies of potential relevance to the review.

Two authors, one with expertise in the topic (LKAF), the second with expertise in the statistical methodology (SM), undertook the search. LKF, SM and AM screened independently titles, abstracts, conferences proceedings, posters and full texts for eligibility. We ensured the avoidance of multiple studies reporting on the same patient population by controlling the duplicate records in a peer review way, including the entire data bases results; we also used standardized, pilot tested forms, together with detailed instructions. Reviewers resolved disagreement through discussion or, if required, by adjudication by a third reviewer (SM).

Assessment of risk bias in included studies:

The included studies were assessed by using Cochrane Collaboration's tool (16) and Quadas-2 (17) for potential source of bias and variation: patient selection bias(study design, patient recruitment, prospective data collection, consecutive patient enrollment)patient variation (demographic features, disease prevalence, disease severity, prior testing) index test bias **(**test review bias, threshold selection) index test variation(observer variation, availability of clinical information, test technology, test execution) reference standard bias (inappropriate reference standard, diagnostic review bias, incorporation bias) reference standard variation (definition of target condition) flow and timing bias (disease progression bias, treatment paradox, partial verification bias, differential verification bias, withdrawals, uninterpretable test results, sample size)

Data synthesis:

Because of differences between studies, it was not possible to pool data, and so a narrative synthesis was prepared. Study population was very heterogeneous, for this reason we classified patients according to the diagnosed disease: endothelial inflammation and atherosclerosis, rheumatoid

arthritis, idiopathic pulmonary fibrosis, Graves' ophthalmopathy, sarcoidosis and other chronic inflammatory diseases.

We found 93 potential studies evaluating radiolabeled SST analogues in chronic inflammatory diseases. After checking for duplicates, 13 papers were immediately excluded, after reading the full text records, there were excluded 40 with reason because did not fulfill the inclusion criteria. We, therefore, analyzed 38 publications, as shown in figure 1. Results are summarized in table 1.

Endothelial inflammation and atherosclerosis

In total, we analyzed 9 studies and excluded 2 for the analysis because they did not complete the inclusion criteria ;7 studies with the use of SRS for endothelial inflammation were included, with a total of 262 patients (Table 1). In details, Tarkin et al. (18), in a descriptive study compared PET with ^{18}F-Fluorodeoxyglucose (FDG) and ^{68}Ga-DOTA-TATE to detect culprit coronary and carotid arteries with acute coronary syndrome, transient ischemic attacks and stroke, and demonstrated best results with ^{68}Ga-DOTA-TATE in the diagnostic accuracy to detect stable and unstable inflamed coronary lesions and vascular inflammation in neighboring coronary and aortic vasculature. When age, total cholesterol, and BMI were evaluated with other relevant clinical factors using multi- variate linear regression, they remained significant predictors of ^{68}Ga-DOTATATE mTBRmax . In patients with TIA or stroke, increase ^{68}Ga-DOTATATE inflammatory signals reliable differentiated between culprit carotid plaques and contralateral non culprit carotid arteries. Ex vivo ^{68}Ga-DOTATATE carotid autoradiography showed high levels of specific ligand binding to SSTR2 receptors. SSTR2 and CD68 mRNA levels were highly correlated within carotid plaque. Carotid SSTR2 and CD68 mRNA levels also showed strong correlation with in vivo ^{68}Ga-DOTATATE TBRmax values measured at the corresponding level in clinical PET images. These data provided both histological and molecular validation of ^{68}Ga-DOTATATE as specific marker of atherosclerotic inflammation. Under QUADAS analysis we

did not find any significant source of risk bias in this report. Malmberg et al. (19) in an observational study in 60 consecutive patients described the differences between ^{68}Ga-DOTA-TOC and ^{64}Cu-DOTA-TATE in detecting atherosclerotic plaques in different vessels, showing better performance of ^{64}Cu-DOTA-TATE explained by the longer half-life which allows for early as well as late PET scanning, even the day after injection. ^{64}Cu has also a substantially shorter positron range than ^{68}Ga, 1 versus 4 mm, rendering it a much better spatial resolution, but a lower positron abundance (20) Uptake of ^{64}Cu-DOTATATE was significantly higher than ^{68}Ga-DOTATOC in the vascular regions both when calculated as maximum and mean uptake. In a multivariate analysis there was a significant association between Framingham risk score and the overall maximum uptake of ^{64}Cu-DOTATATE using SUV as well as target-to-background ratio, whereas no association was found with ^{68}Ga-DOTATOC. The association of risk factors and maximum SUV of ^{64}Cu-DOTATATE was found driven by body mass index, smoking, diabetes, and coronary calcium score suggesting the potential use of this radiotracer as noninvasive biomarker of cardiovascular risk. According to QUADAS 2 analysis we considered the reference test the main source of risk of bias, because it did not use the anatomopathological evidence to validate the results on ^{68}Ga-DOTATOC but the calcium score on CT. Simon Wan et al. (21) conducted a descriptive study with 20 patients with recent stenotic carotid events and described the findings of ^{68}Ga-DOTA-TATE in carotid plaques versus histology. Surprisingly they did not find any significant uptake of ^{68}GaDOTATOC in the plaques neither activated macrophages expressing SSTR2 and they could not support the use of ^{68}GaDOTATOC for evaluating the inflamed plaque; the source of bias in this report, (misclassification explained by heterogeneity of time between carotid event, research PET, and endarterectomy) could maybe explained the results. In QUADAS 2 analysis this source of bias corresponds to flow and timing. Pedersen et al. (22) in a population of 10 patients with clinical symptoms of stroke or transient ischemic attack, described the findings of ^{64}Cu-DOTA-TATE uptake in atherosclerotic

plaques. ^{64}Cu-DOTA-TATE uptake was significantly higher in symptomatic plaques and correlated with CD163 expression by activated macrophages at histology. We did not find any important source of risk of bias in this report according to QUADAS analysis. Xian Li et al. (23) in a retrospective series of 16 patients, analyzed simultaneously ^{18}F-FDG and ^{68}Ga-DOTA-TATE PET/CT and showed significantly increased uptake in the fibrotic and vulnerable atherosclerotic plaques compared to normal coronary arteries, suggesting a potential role of this tracer for molecular assessment of coronary artery disease. They found higher values of focal uptake with ^{68}GaDOTATATE in patients with high cardiovascular risk and significant correlation between the mean uptake of both tracers and the patient´s score of risk factor. We found that the reference test and the index test were source of risk of bias in QUADAS 2 analysis. Mojtahedi et al. (24) generated a preliminary data by using ^{68}Ga-DOTATATE PET/CT, with in vulnerable or fibrotic atherosclerotic plaques in the coronary arteries. In a population of 44 patients with neuroendocrine tumors (NET) who underwent ^{68}Ga-DOTATATE PET/CT, they found that the mean TBR value in the normal group was 1.345 ± 0.58 while the mean TBR value in the fibrotic plaque group was 1.752 ± 1.50 ($p= 0.0043$) and in atherosclerotic plaques group was 2.043 ± 1.76, $p<0.0001$). There was a significant correlation ($p=0.0026$) between ^{68}Ga-DOTATATE uptake and the progression to formation of atherosclerotic plaques, based on coronary CT calcium score, HU. Those findings suggested the potential of ^{68}Ga-DOTATATE PET/C for molecular assessment of coronary artery disease. According to QUADAS 2 analysis we considered as potential source of risk of bias the reference standard they used for validate the ^{68}Ga-DOTATATE PET results. Rominger et al. (25) in a descriptive analysis of 70 consecutive patients correlated ^{68}Ga-DOTATATE uptake in the left anterior descending coronary artery with the presence of calcified plaques and cardiovascular risk factors. Higher uptake lead distinguish between patients with and without coronary calcifications. As in the abovementioned reports the main source of bias of this

series in QUADAS 2 analysis was related to the reference standard they used to validate the ^{68}Ga-DOTATATE results.

It is well known that monocytes and macrophages, being the first inflammatory cells associated with vascular inflammation, play an important role in the pathogenesis of atherosclerosis, contributing to necrotic core formation, fibrous cap thinning, and plaque vulnerability (26). These cells express SSTR-2 on the cell surface in cultures (27) and therefore ^{68}Ga-DOTA-TATE uptake was thought to have the potential to be a surrogate marker of inflammation to study plaque biology (28). Taking together all published data, it is likely that there are complex relationships between the stage of plaque evolution (chronic vs acute), macrophage density and activity, SSTR subtype expression, and ^{18}F-FDG and ^{68}Ga-DOTA-TATE uptake. Evidences, so far, show a tendency to confirm the utility of SRS with ^{68}Ga-DOTA-TOC to detect high risk, vulnerable, atherosclerotic plaques and an interesting correlation with risk factors, with the added value of TOC (specific for inflammatory cells only provides an in vivo information on the vulnerability of plaques) in contrast to FDG (global inflammatory burden but nonspecific and does not provide a relevant histopathological information) and to ^{FL}-Na and to calcium score (29).

Rheumatoid Arthritis

In total, 11 publications were analyzed, but just 2 clinical studies were included with in total 36 patients (Table 1), since most were reviews, case reports or in-vitro or animal studies. Anzola et al. (30) conducted a pilot study to evaluate the uptake pattern of ^{99m}Tc-EDDA/HYNIC-TOC in the joints of 18 patients with rheumatoid arthritis refractory to treatment. They showed high uptake of the molecule in all evaluated joints and also in the salivary glands of some patients as possible evidence of Sjögren's Syndrome. There was a statistically significant reduction in uptake after treatment with anti-TNFα antibodies in all patients. The authors concluded that this scan can be useful to select and monitor RA patients for therapy with anti-TNFα antibodies. In QUADAS 2 analysis we found as potential source of risk of bias the index test because the lack of standardized method to evaluate the results. Vanhagen et al. (31)

reported the results of 14 patients with RA and 4 with osteoarthritis using ^{111}In-octreotide. They reported sensitivity (uptake of the tracer in swollen and painful joints) of 76% in RA patients and of 20% in osteoarthritis. As in the abovementioned series we found lack of a standardized method to evaluate the results and weakness in the reference test used to compare the results. (QUADAS 2, reference and index test source of bias)

Synovia of patients with active rheumatoid arthritis expresses a high density SSTR (32). The experience published in these two clinical papers, shows the potential of SRS to localize and identify sites of active inflammation in joints, both symptomatic and asymptomatic, and extra-articular involvement as in salivary glands. The information provided by SRS is different from other imaging modalities and provides in vivo histopathological information on the activity of cell-mediated inflammation. This information has an important impact for therapy decision- making and therapy follow-up as also appears in other studies (33).

Idiopathic pulmonary fibrosis

In total, 7 studies were analyzed, but only 3 studies could be included with in total 51 patients (Table 1). Ambrossini et al. (34) in an observational study evaluated the potential role of ^{68}Ga-DOTANOC PET/CT in patients with idiopatic pulmonary fibrosis (IPF) and non-specific interstitial pulmonary pneumonia (NSIP). ^{68}Ga-DOTANOC PET/CT findings were compared with HRCT results. In IPF patients, ^{68}Ga-DOTA-NOC uptake appeared as a typical subpleural and peripheral distribution involving both lung fields predominantly at the bases. Areas of ^{68}Ga-DOTA-NOC uptake directly corresponded to pathologic areas on HRCT. In contrast, ^{68}Ga-DOTANOC uptake was faint in NSIP patients and undetectable in healthy control subjects. No significant association was documented in NSIP cases. According to QUADAS 2 analysis, we found as source of potential risk of bias the index test because they did not use a previous standardized method to evaluate the results and they did not confirm the findings under histopathological analysis.

Lebtahi et al. (35) investigated the expression in vivo of SSTR in the lungs of 11 patients with IPF, and 6 patients with pulmonary fibrosis associated with systemic sclerosis by using ^{111}In-octreotide scintigraphy and compared the results with 19 control patients. They reported higher uptake in all 11 IPF patients and in 4 of 6 systemic sclerosis patients related to normal control population. Increased uptake correlated significantly with different lung function and cytological tests. As in the abovementioned series according to QUADAS 2 analysis, we found as potential source of risk of bias the index test and the reference test explained by the same conditions argued before.

Win Thida et al. (36) analyzed in an observational study 26 patients with diffuse parenchymal lung disease (10 patients with idiopathic pulmonary fibrosis, 12 with nonspecific interstitial pneumonia and 4 with other forms of interstitial lung disease), by using ^{68}Ga-DOTA-TATE and ^{18}F-FDG by combined PET and HRCT. All patients demonstrated increased pulmonary signal with both ^{68}Ga-DOTA-TATE and ^{18}-F-FDG.

The experience reported in this review shows the potential utility of the SSTR scintigraphy to localize and to detect the active inflammation mediated by an immunological response in idiopathic pulmonary fibrosis. In QUADAS 2 analysis, we found as source of risk of bias the reference test and index test explained by the same reasons argued above.

Graves' ophthalmopathy

In total 33 papers were reviewed, 14 studies with in total 451 patients were included (Table 1). Aguirre-Balsalobre et al. (37) in a series of 18 patients affected by thyroid orbitopathy with Graves diseases by using a semiquantitative analysis of the images with ^{111}In-Pentreotide, demonstrated how patients with higher scores had better response to corticosteroid therapy; according to QUADAS 2 analysis, the source of bias of this experience was related to the index test because lack of standardized method. Colao et al. (38), Gerdin et al. (39), Kahaly et al. (40), Kahaly et al. (41), Krassas et al. (42), Krassas et al. (43), Moncayo et al. (44), Nocaudie et al. (45), Postema et al.

(46), in similar study designs by using ^{111}In Octreotide described how patients with active orbitopathy in Graves' disease had higher uptake of the tracer in the orbits, and how this finding was helpful to discriminate active disease vs non active disease and how it helped to choice better the candidates to immunotherapy, radiotherapy or somatostatin analogues therapy. In those series it was possible to observe how patients with higher uptake in orbits responded well to the installed treatment. The common source of bias for the abovementioned authors according to QUADAS 2 analysis was related to the index test and although none of the authors used a standardized method to analyze the images, the results were not only similar but promissory; more over in Postema´s report it was seen a high frequency of source of risk bias related to patient selection. In Postema´s series, it was also found the patient selection as source of risk of bias. (Fig.2).

Burggasser et al. (47) in a series of 44 consecutive patients, by using ^{99m}TcP-829, demonstrated statistically differences in the orbital uptake between control and disease patients and between active and inactive orbitopathy, in Graves' disease patients with orbitopathy. Even though they used two different methods to analyze the images, we considered the index test as source of bias according to QUADAS 2 analysis. Huang Sung et al. (48) by using ^{99m}Tc HYNIC-TOC reported similar results of the above authors with the same previous described source of bias.

Rong Zhao et al in a prospective study (49) by using SPECT/CT system as novel technique ,investigated the predictive role of the orbital somatostatin receptor scintigraphy with ^{99m}Tc-EDDA/HYNIC-TOC (^{99m}Tc-TOC) to detect clinical stage of Graves' ophthalmopathy (GO) and the response to corticosteroid therapy in a sample of 46 patients with GO and four volunteers without eye disease. The treatment effect was evaluated both by the orbital ^{99m}Tc-TOC uptake and NOSPECS. Orbital images were quantified by region of interest (ROI) analysis. Semi-quantitative evaluation of retrobulbar uptake was performed with irregular ROIs which were placed over the orbits (O) and the reference area over the occipital area (OC). This prospective study

demonstrated marked orbital accumulation of ^{99m}Tc-TOC in 22 GO patients, showing higher activity score and clinical improvement; They demonstrated good correlation of orbital ^{99m}Tc-TOC scintigraphy with CAS. The value of this study was to identify the anatomical structures depicted with ^{99m}Tc-TOC in patients with GO by means of SPECT and CT image fusion analysis. Thus, in the absence of histology, because of a favorable target-to-background ratio and fusion imaging of SPECT/CT in this study, orbital ^{99m}Tc-TOC fusion imaging was able to determine the pathological phase of Graves' disease, giving a high positive scan in the active early phase and a low positive or negative scan in the stable end phase of the disease. We consider that the analysis of the images under hybrid images diminishes the risk of bias related de index test. (QUADAS 2).

Lincke et al. (50) is the only report so far that used PET study in thyroid- related pathologies. He compared the characteristics of uptake between ^{111}In-Pentreotide and ^{68}Ga-DOTA-TOC in normal and pathologically altered thyroid glands and found that both tracers showed higher uptake in active Hashimoto and Graves' disease most likely caused by SSTR expressing lymphocytes in the thyroid tissue; however, the physiologic or pathophysiologic relevance of the increased In-111 pentetreotide and Ga-68 DOTATOC uptake in normal thyroid glands, hot and cold nodules, and goiters and nodular thyroids remain to be determined. According to QUADAS 2 analysis in this report the index test and the reference test were considered as unclear source of bias.

During the last 20 years, it demonstrated the utility of SSTRs scintigraphy identifying active orbitopathy in Graves exophtalmopathy. No matter as a common source of bias in those series it was found to be lacking of validated method to evaluate the SSTR scintigraphies, all the authors reported similar results showing scintigraphic patterns consistently with high uptake of the molecule in the periorbitary zone as evidence of active disease with prognostic implications.

Sarcoidosis and other chronic inflammatory diseases

As far as sarcoidosis is concerned, were reviewed 18 publications, of which 6 were excluded (Table 1). Lapa et al. (51) in an observational study conducted a pilot prospective study in 6 patients with active peri-/myocarditis and 6 with sub-acute myocardial infarction ,who underwent SSTR-PET/CT , ^{68}Ga-DOTA-TOC and cardiac MRI within 3-10 days after onset of symptoms. They compared the results under semiquantitative analysis with 12 oncologic patients with no history of coronary artery disease. In patients with clinical evidence of myocarditis, PET/CT returned 26 positive segments and MRI 13 segments, respectively. In patients with myocardial infarction, there were 29 ^{68}Ga-DOTA-TOC positive segments and 31 abnormal segments in MRI. On a head-to-head comparison, SSTR-PET and MRI were concordantly positive in 36 segments (76.6%). Nineteen segments were SSTR-PET positive and MRI-negative (9.3%; 19/204), and 11 SSTR-PET negative and MRI-positive (5.4%; 11/204). Both modalities returned negative results in 138 segments, thus leading to an overall concordance of 85.3%. Agreement of the modalities was higher in patients with myocardial infarctions. SUV mean and SUV max were significantly higher in the infarcted/ inflamed myocardium as compared to remote myocardium or the left ventricular (LV) cavity.
We did not find any relevant risk of bias according to QUADAS 2 analysis (Fig 3).The overall concordance of the 2 modalities was 96.1%. Kwekkeboom et al. (52) in a cross sectional study in 46 patients with known mediastinal, hilar and interstitial sarcoidosis, by using SRS with ^{111}In-pentetreotide showed positive results in 36 out of 37 patients with known pathology, and in 7 with normal X-rays. In 13 patients who had no evidence of extrapulmonary disease involvement with physical examination and conventional imaging, somatostatin receptor imaging revealed abnormal uptake of radioactivity outside the chest. Neither the degree of radioactive accumulation nor a specific pattern of pathological uptake was correlated with disease severity or clinical course. They repeated de scintigraphy on 13 patients, 5 of 6 patients who showed X-Rays improvement, the SSTRs also improved . Two of 5 patients

whose X-rays did not improve, lung function and SSTRs improved. They could not conclude that pentetreotide scintigraphy has a role in predicting prognosis or clinical course in patients with sarcoidosis. According to QUADAS 2 analysis, the source of risk of bias in this series was related to the lack of an standardized method to evaluate the results in the index test, and also we found a low concern related to the reference test used in some situations. Lebtahi et al. (53) in an observational study demonstrated the superiority of ^{111}In Pentetreotide compared with ^{67}Ga scintigraphy in the evaluation of pulmonary and extrapulmonary involvement in 18 patients with proven sarcoidosis especially in the hilar or mediastinal area. Nine were or recently had been receiving steroid therapy at the time of the examination. Gallium scintigraphy found abnormalities in 89% of patients and detected 65% of the clinically involved sites. SRS found abnormalities in 100% of patients and detected 83% of clinically involved sites. Of the 9 treated patients, gallium scintigraphy found abnormalities in 78%, detecting 59% of the clinically involved sites whereas SRS found abnormalities in 100%, detecting 82% of the clinically involved sites. SRS images were consistently better than gallium images, with well-delineated lesions, especially in the hilar or mediastinal area, on either planar or SPECT images. They concluded that SSTRs seems to be a promising alternative to gallium scintigraphy for evaluating the extent of sarcoidosis, detects significantly more sites of sarcoidosis involvement with lesion contrast significantly higher than with gallium scintigraphy, especially for lung and mediastinal involvement. Despite the good results reported we found as potential source of bias in QUADAS 2 analysis the lack of standardized method to evaluate the images on ^{111}In pentetreotide and also the lack of a well- defined reference test. Kamphuis et al. (54) in a retrospective study evaluated the additive value of SRS scintigraphy with ^{111}In pentreotide in the clinical evaluation of sarcoidosis and compared the results with chest X-ray and CT. In both histologically proven and unproven sarcoidosis, in all but one SSTR uptake was demonstrated. In the thoracic region SRS increased the yield with 36% and 32% in comparison with X-ray and CT, respectively. In the

histologically proven group, there were no negative SRS results, and the SRS increased the yield for thoracic localization in 30% and 14% of the patients in comparison with X-ray and CT, respectively. As in the abovementioned series, although the results reported were encouraging, we found in QUADAS 2 analysis that the lack of a standardized method for evaluating the SRS scintigraphy with ^{111}In pentreotide was a source of potential bias as well the reference test was source of potential risk of bias. Gormsen et al. (55) in a pilot study in 19 patients with suspected cardiac sarcoidosis compared the diagnostic accuracy and inter-observer variability of ^{68}Ga-DOTANOC vs ^{18}F-FDG PET/CT. Cardiac sarcoidosis (CS) was diagnosed in 3/19 patients. By consensus, 11/19 ^{18}F-FDG scans were rated as inconclusive, resulting in low agreement among reviewers (Fleiss combined kappa 0.27) and correspondingly poor diagnostic accuracy; For ^{68}Ga-DOTANOC, no scan was rated as inconclusive but it was reported a poor interobserver agreement (Fleiss combined kappa 0.46). The sensitivity of ^{18}F-FDG PET for diagnosing CS was 33 %, specificity was 88 %, PPV was 33 %, NPV was 88 %, with 79% of diagnostic accuracy; In contrast for ^{68}Ga-DOTANOC, and accuracy was 100 %. ^{68}Ga-DOTANOC PET/CT looks very promising as an alternative CS PET tracer. When we analyzed under QUADAS 2 protocol we found in the index test and reference test the potential source of risk of bias.

Nobashi et al. (56) in a descriptive study compared the utility of ^{68}Ga-DOTA-TOC with conventional ^{67}Ga-citrate in the visualization of active sarcoidosis and correlated quantitative parameters on ^{68}Ga-DOTA-TOC PET/CT with clinical data. Twenty patients with sarcoidosis underwent both studies. The diagnosis was confirmed in 12 patients histologically and in the other 8 on clinical and paraclinical data. Active lesions were identified by visual interpretation when focal increases in radioactivity were higher than the normal biodistribution of tracers. Quantitative analysis on ^{68}Ga-DOTATOC was made by using SUVmax values. DOTATOC-PET/CT showed abnormal findings in 19 patients, whereas ^{67}Ga showed abnormal findings in 17. DOTATOC was superior than ^{67}Ga identifying lymph nodes ($p<0.046$). SUVmax in each organ

did not show statistical difference between involved organs, however the average SUVmax of lymph nodes was highest among the involved organs. It was not seen as statistically different in SUVmax between patients with and without symptoms. Active lesion volumes calculated by DOTATOC-PET moderately correlated with serum ACE concentrations. ^{68}Ga-DOTATOC-PET was superior to ^{67}Ga in visualizing lesions in the uvea and muscle as well in the lymph nodes. As source of bias there was partial verification bias because the lack of in vitro analysis of somatostin receptor in the lesions and because not all patients were diagnosed by the same reference standard. There is patient variation bias in some patients who were receiving treatment for the clinical condition in the same time when there were acquired the studies. According to QUADAS 2 analysis, the potential source of bias in this series relied on the index test and reference standard explained by the same arguments in the above references.

Piotrowoski et al. (57) in an observational study, evaluated 32 patients with sarcoidosis and used ^{99m}Tc-HYNIC-TOC scintigraphy as a reference method to evaluate the clinical usefulness of traditional markers, such as angiotensin-converting enzyme (SACE), C-reactive protein (CRP), serum calcium(S-Ca^{2+} level, and 24-hour Urinary-Ca^{2+}, bronchoalveolar lavage fluid (BALF lymphocytes) as well as of a novel marker of lipid peroxidation-8-IP in exhaled breath condensate (EBC 8-IP). They divided the patients according to the ^{99m}Tc-HYNIC-TOC results in two groups: Grade 1 (20 patients) when the sintigraphy was positive for an abnormal uptake in thorax and grade 0 (12 patients) when the scan was negative. The only significant difference between scintigraphic negative vs positive results was found in the level of EBC 8-IP, unfortunately, this marker is not specific for sarcoidosis. Furthermore, it observed a trend towards higher levels of SACE in the group with positive radiotracer uptake. Although the tendency was noticed towards higher percentage in patients with positive scintigraphy results, the statistical significance was not reached. It was not possible to demonstrate the capacity of any laboratory test to estimate the intensity of the inflammatory process.

The SSRTs could discriminate between positive and negative studies but further studies are needed to find the utility of those results. The reference standard was the most frequent source of bias and applicability concern in QUADAS analysis.

SRS has been proposed also for studying other chronic inflammatory conditions. In particular in histiocytocis, tuberculosis, cardiac allograft rejection and small vessel vasculitis.
Weinmann et al. (58) evaluated 13 patients with histiocytosis. Lung uptake of ^{111}In-pentreotride in a visually and semiquantitative analysis was statistically significantly higher in patients compared to controls. This report showed concern as potential source of bias in the reference and index test.
Oztürk et al. (59) described three similar cases: 2 patients with pulmonary sarcoidosis and one with tuberculosis by ^{111}In-pentreotide scintigraphy; this report generated in QUADAS 2 analysis concern about the source of risk of bias in the three items contained in the evaluation: patient selection, index test and reference standard.
Vanhagen et al (60) reported in a consecutive series of 20 patients the utility of ^{111}In-octreotide localizing sites of granuloma infiltration in patients with sarcoidosis and tuberculosis. In vitro autoradiography of fresh tissue biopsies showed bonding of the molecule at sites that were microscopically identified as granulomatous disease. The major contribution of this study was that this scintigraphy procedure demonstrated the expression of somatostatin receptor in such kind of granulomas. Under QUADAS 2 analysis we found a potential risk of bias the index test because of the lack of a standardized method to evaluate the results.
Aparici et al. (61) conducted a prospective study in 13 patients with suspected cardiac allograft rejection to assess the feasibility of SRS with ^{111}In-pentreotide to target activated lymphocytes in transplanted heart as a possible early marker of rejection. A high cardiac uptake was observed in 8 patients: 3 had an acute rejection and 5 had mild or no rejection. However, in 4 of the 5 patients with no rejection at time of study, a biopsy, performed 1 week later, demonstrated a significant rejection requiring treatment. These preliminary

results indicate the feasibility of targeting activated lymphocytes for the detection of cardiac allograft rejection and suggest a possible predictive role of SRS. These preliminary results indicate the feasibility of targeting activated lymphocytes with somatostatin receptor imaging in the detection of cardiac allograft rejection and suggest a possible predictive role of SRS. Index test generated in QUADAS analysis the high concern as source of bias and eligibility concern. The method used to evaluate the pattern uptake was not validated previously.

Neumann et al. (62) by using ^{111} In-pentreotide analyzed 32 consecutive patients, with ANCA-associated small vessel disease (AASV). Disease activity was evaluated with the Birmingham Vasculitis Activity Score (BVAS). For pulmonary AASV, SRS showed a sensitivity of 86% and a specificity of 96% with a positive predictive value of 97% for active disease. False negative scans were seen in patients under immunosuppressive therapy. In patients with ear/nose/throat involvement, SRS showed a sensitivity of 68% and a specificity of 100% with a positive predictive value of 100%. In patients who responded to therapy and went into full remission, repeat SSTR scintigraphy demonstrated the absence of previously present tracer accumulation; patients with aggressive disease who responded poorly to immunosuppressive therapy remained positive at repeat scintigraphy. Immunohistochemical analysis for SSTR2 and SSTR3 was performed on three open lung biopsies in active disease obtained from two patients with Wegener's granulomatosis (WG) and one patient with microscopic polyangiitis as well as on nasal and lung tissue of one autopsy case with WG. All specimens demonstrated STR2 y and STR3 expression on monocytes-macrophages and giant cells surrounding granulomas and occasionally in the center of the granulomatous reactions. The reference standard generated in QUADAS analysis high concern as source of bias and applicability concern, because the immunohistochemical analysis was obtained just in three patients.

Reported evidence so far shows the utility of ^{68}Ga-DOTA-TOC for detecting sarcoidosis lesions, especially for lymph nodes, uvea and muscles, as well as

for vasculitis and other chronic inflammatory diseases. In particular, the results obtained for monitoring cardiac allografts open a new important clinical application of SRS to early detect lymphocytic infiltration in transplanted tissues and to monitor the effect of therapies in preventing rejection.

Discussion

This is the first systematic review performed about the usefulness of SRS (both SPECT and PET) to diagnose chronic inflammatory diseases. The role of somatostatin as mediator in inflammatory processes and how different immunological cells express SSTRs is well known. The possibility to specifically target the SSTRs by molecular imaging gave us the opportunity to visualize active inflammation in a variety of inflammatory disorders.

In general, based on the results of this systematic review, we found that SRS has a large potential to be used as diagnostic tool in patients suspected to have chronic inflammatory diseases. The evidence reported until now is supported mainly by observational studies that were designed to investigate different groups of chronic inflammatory conditions: we found a wide heterogeneity in used protocols, in studied conditions, and in the studied population. Furthermore, we observed that in almost every study a validated and standard method to analyze the images was lacking, a condition that became the most important source of bias.

The most frequent pathology evaluated by PET was the endothelial inflammation and vulnerable plaques, where promising correlations between quantitative uptake and histopathology were found, emphasizing the role of SRS for this inflammatory condition. Monocytes and macrophages were the first inflammatory cells to be associated with atherosclerosis. They are believed to play important roles in its pathogenesis, contributing to necrotic core formation and fibrous cap thinning in advanced atherosclerosis, features thought to confer vulnerability (26) It is known that human macrophages express sst2 on their cell surface in cell culture (27). ^{68}Ga-DOTATATE, a specific sst2 receptor agonist, therefore was thought to have the potential to

be a surrogate marker of inflammation to study plaque biology(28) . Taken together, it is likely that there are complex relationships between stage of plaque evolution (chronic vs. acute), macrophage density and activity, somatostatin receptor subtype expression, ^{18}F-FDG, and somatostatin receptor PET signal. The today evidence shows a high tendency to confirm the utility of the SSTRs ^{68}GaDOTATOC to detect the high risk atherosclerotic plaque and its interesting correlation with risk factors. Studies are needed to confirm those finding with better designs which enables to choose better the study population, to calculate a good sample size and to validate the findings with good references tests.

The peripheral nervous system and its neuropeptidergic pathways may play an important role in the pathogenesis and development of rheumatoid arthritis. Somatostatin via its receptors has shown to be implicated in inflammatory diseases and particularly it is known how the synovium in active rheumatoid arthritis express a high density of somatostatin receptors (32). The experience published in these papers although lacks of a rigorous methodology, shows the potential of radiolabeled somatostatin receptors to localize and identify sites of active inflammation in joints and extra articular involvement and to identify the patients who could benefit of the therapy. In this setting it will be necessary to conduct rigorous prospective studies to validate the methodology to make an accurate approach in arthritis patients.

Different radiotracers were used in patients with Idiopathic Pulmonary Fibrosis (IPF). Ambrosini reported results with ^{68}Ga-DOTANOC, which presents the broader SSTR-subtype affinity (63), a favorable dosimetry, and no uptake in the intact lung (64); recent preclinical evidence demonstrated SSTR expression on fibroblasts of both murine models of IPF and human tissue samples from IPF patients (65). Although ^{68}Ga-DOTANOC binding sites within the lungs cannot be precisely localized without direct sampling, the evidence that tracer uptake was observed in IPF cases allows one to speculate that PET with ^{68}Ga-DOTANOC might be useful to identify SSTR overexpression in

this patient subgroup. In Ambrosini´s report areas of ^{68}Ga-DOTANOC uptake directly corresponded to pathologic areas on HRCT and the preliminary data supported the hypothesis that SSTR is over expressed in the lungs of IPF patients. Lebtahi showed an interesting correlation between STRR scintigraphy findings with ^{111}In-Octreotide in IPF patients and functional pulmonary tests. Win Thida reported results with ^{68}Ga-DOTATATE and ^{18}F-FDG with similar pattern of distribution with both tracers. The experience above mentioned, although has methodological deficiencies showed clearly the potential utility of STRRs imaging detecting active disease in pulmonary fibrosis and opening the possibility to include this technique not only for controlling the response to the therapy, but selecting those who might benefit from somatostatin analog treatment or combined approaches in which ^{68}Ga-DOTANOC may function as a carrier for specific antifibrotic drugs or cytotoxic isotopes.

The evidence that ^{111}In-DTPA octreotide is highly concentrated in the orbits of patients with Graves' ophthalmopathy indicated new frontiers in the diagnostic work-up of the disease (46). Somatostatin receptors, especially SSTR-2 and SSTR-5 subtypes, with high affinity for octreotide (66). are found in retro-ocular muscles and retrobulbar fat of Graves' ophthalmopathy .During the active phase of disease, activated lymphocytes also express somatostatin receptors. Several studies (39,41) have already shown that scintigraphy with ^{111}In-pentetreotide can reveal massive orbital uptake of this analogue of somatostatin in cases of thyroid-associated ophthalmopathy, in contrast to other non-thyroid causes of exophthalmia. Because of the kinetics of the radiotracer and because of the increased blood flow occurring in active thyroid-associated ophthalmopathy, the early pentetreotide accumulation is high and, according to Kahaly et al (41), could be a sensitive criterion of active disease. Thyroid-associated ophthalmopathy (TAO), is one of the most difficult autoimmune disorders to investigate; The clinical definition of the activity of the ophthalmopathy at the initial presentation is crucial to the identification of the

correct therapeutic approach (67). The treatment of TAO is based on immunosuppressive therapy, such as the use of corticosteroids or radiation, or surgical therapy (68). Immunosuppression is considered beneficial only in the early active stage, whereas surgery represents the treatment for the end stage of the disease. On the basis of this evidence, a new approach, using labelled somatostatin analogues intended to highlight the presence of activated T lymphocytes in orbital tissue, was attempted in order to identify the early stage of TAO, which should be particularly sensitive to immunosuppressive treatment (69). Although the evidence to date is characterized by series with lack of standardized methods for evaluating the scintigraphic findings, there is a common trend to demonstrate a potentially relevant role for SRS in the pretreatment evaluation of TAO (70), showing for instance a positive correlation between clinical activity score and ^{111}In-Octreotide uptake in patients with TAO (40,46), Similarly, others (39) reported that visual semi-quantitative analysis of 4 h/24 h planar images during SRS was correlated with the ophthalmologic progression. As the presence of activated lymphocytes in the orbit represents the first requisite for the effectiveness of corticosteroid therapy and as it can be revealed by SRS, recently it was attempted this approach in patients with TAO. The results of orbital ^{111}In-Octreotide uptake predicted the response to corticosteroid therapy in these patients (38). Nocaudie et al (45) found that all patients with severe thyroid ophthalmopathy with positive ^{111}In-DTPA Octreotide results showed clinical improvement at 6 months, whereas patients with negative SRS results had not improved. Although a larger group of patients should be investigated before definitive conclusions can be drawn, SRS seems to be a useful method for predicting the clinical response to immunosuppressive treatment in patients with TAO. This might avoid the use of corticosteroid therapy in patients for whom it would have only adverse effects, and might delay surgical treatment for those patients who could benefit from other kinds of medical therapy. Furthermore some authors (41,44) demonstrated how ^{111}In Octreotide uptake in both orbits was significantly lower after corticosteroid and irradiation treatment (41) and

after immunosuppressive therapy (43).

The experience reported in sarcoidosis has the biggest sample of patients (275). In cardiac sarcoidosis, SSTRs might allow for direct assessment of disease activity, especially in the course of treatment. While the current gold standard MRI depicts structural changes like cardiac damage and scarring and edema with the highest spatial resolution, ^{68}Ga-DOTATOC uptake may directly reflect the underlying immunological cell activity. In future, given the complementary nature of PET and MRI signals, the combination of the two may be the optimal diagnostic approach, preferably by integrated MRI/PET. Additionally, whereas a recent study (56) comparing ^{68}Ga-DOTANOC and ^{18}F-FDG PET/CT has demonstrated encouraging diagnostic accuracy for SSTR-directed PET, the prognostic value of ^{68}Ga-DOTATOC PET/CT, especially in comparison to ^{18}F-FDG has to be clarified in future trials. Sarcoidosis is a multisystem granulomatous disorder, most frequently involving the lungs, skin, or eyes. Somatostatin receptor scintigraphy (SRS) can visualize sarcoid granulomas through binding of a radionuclide-coupled somatostatin analog to somatostatin receptors that are expressed in sarcoidosis. Somatostatin receptor subtype 2 (SST2) is highly expressed in sarcoid granulomas and used as a substrate for somatostatin receptor scintigraphy (SRS) with ^{111} In-DTPA octreotide and the pendant in PET imaging with ^{68}Gallium-labeled somatostatin analogues (71,72). The use of SSTR-targeted radiotracers to assess sarcoidosis disease activity is not a novel idea. Giant cells, epithelioid cells, and lymphocytes constitute the bulk of active inflammatory cells in sarcoid granulomas and express SSTRs abundantly (56). ^{68}Ga-DOTANOC PET/CT detected significantly more sites than did ^{67}Gallium scintigraphy (P= 0.001), especially for thoracic and central nervous system involvement, and appeared more accurate for evaluation of disease activity. ^{68} GaDOTATOC-PET was also used and was superior to ^{67}Ga in visualizing lesions in the uvea and muscle as well in the lymph nodes in patients with proven sarcoidosis. Three reports (52-54) demonstrated the utility of ^{111}In-pentetreotide detecting extrapulmonary disease involvement in sarcoidosis and its superiority related

to ^{67}Gallium nevertheless neither the degree of radioactive accumulation nor a specific pattern of pathological uptake was correlated with disease severity or clinical course, and the SSTRs augmented the yield for thoracic localization in 30% and 14% of the patients for X rays and CT respectively. There is one report (57) that used ^{99m}TcHYNIC-TOC in sarcoidosis patients and compare the results with laboratory tests and although the SSTRs clearly could discriminate between positive and negative studies further studies are needed to find the utility of those results. The evidence about the utility of radiolabeled somatostatin receptor in granulomatous diseases is weak, and although in the found references the authors have reported increase uptake of the tracer in the inflammatory focus and visualization of granuloma sites with ^{111}In-pentetreotide, the use of radiolabeled somatostatin receptor in patients with granulomatous disease, such as sarcoidosis, tuberculosis, and Wegener's granulomatosis (73) has also been previously reported; further studies would be important to validate the technique tested with good reference tests and with new radiolabeled somatostatin receptors as with ^{68}Ga tracers. The characteristics of ^{67}Gallium, as far as lower lesion contrast, the physiological uptake and the photon energy are overcome by the characteristics of SSTRs, making it a promising alternative to scintigraphy for evaluating the extend of sarcoidosis (53).

The invasive nature of endomyocardial biopsy has led to a search for alternative diagnostic modalities for the detection of cardiac allograft rejection. The rejection process usually presents with lymphocyte infiltration with or without myocyte necrosis, which indicates the severity of cardiac allograft rejection and the necessity of treatment. Activated lymphocytes express somatostatin receptors; thus somatostatin receptor imaging could be used to target them. The published experience shows the attractive possibility to screen cardiac graft rejection. It is a series of cases with the limitation of the radioligand labeled with ^{111}In, with a source of bias, misclassification, explained by the technical limitations of the index test, not only because the lack of a validated method to evaluate the findings but the image

characteristics of the ^{111}In as radioligand. It would be interesting conduct a prospective study by using ^{68}Ga compounds.

The upper and lower respiratory tract are common targets in antineutrophil cytoplasmic antibody (ANCA)-associated small vessel diseases (AASV) such as Wegener's granulomatosis (WG), microscopic polyangiitis (MPA) and the Churg–Strauss syndrome (CSS). Although ANCAs have been proven as an important diagnostic tool (74) it has to be emphasized that, especially in limited AASV, a negative ANCA result does not exclude the diagnosis of active WG or MPA (75). In AASV, activated T cells are believed to play a central role in pathogenesis (76) and the prominence of T cells and monocyte– macrophages has been demonstrated in lung, (77) ear, nose and throat (ENT) (78) and kidney specimens of active AASV (79). The promising results of Neuman´s experience highlight the potential value of SSTR scintigraphy as a non-invasive diagnostic procedure that could detect active disease early in the course of (AASV) and register disease extent, reflecting also response to treatment.

Conclusions

The evidence summarized in this systematic review highlights the promising results of the potential value of SSTR scintigraphy to detect active disease in different inflammatory conditions mediated by activated somatostatin receptors; although the lack of standardized methods for evaluating the parameters in the index test was the most common characteristic in the majority of the series, there was a clear trend to report positive results related to activated somatostatin receptors in the different organs affected by the inflammatory conditions. In this review, the most solid results had to do with the potential in the atherosclerotic plaque field where undoubtedly the contribution of nuclear medicine by PET/CT systems provided important information about the prediction of acute cardiac events. Because of the methodological limitations in the other series, the results must be rigorously

analyzed without ignoring the probity of the molecule. As a future perspective, it is meaningful to encourage the scientific community to advocate the conduction of large and robust prospective studies with the intention of validating the technique in different scenarios; henceforth, considering the inclusion of this tool in different decision-making trees for diagnostic and prognostic purposes.

Compliance with Ethical Standards

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Figure legend

Figure 1
Prisma flowchart of article selection.

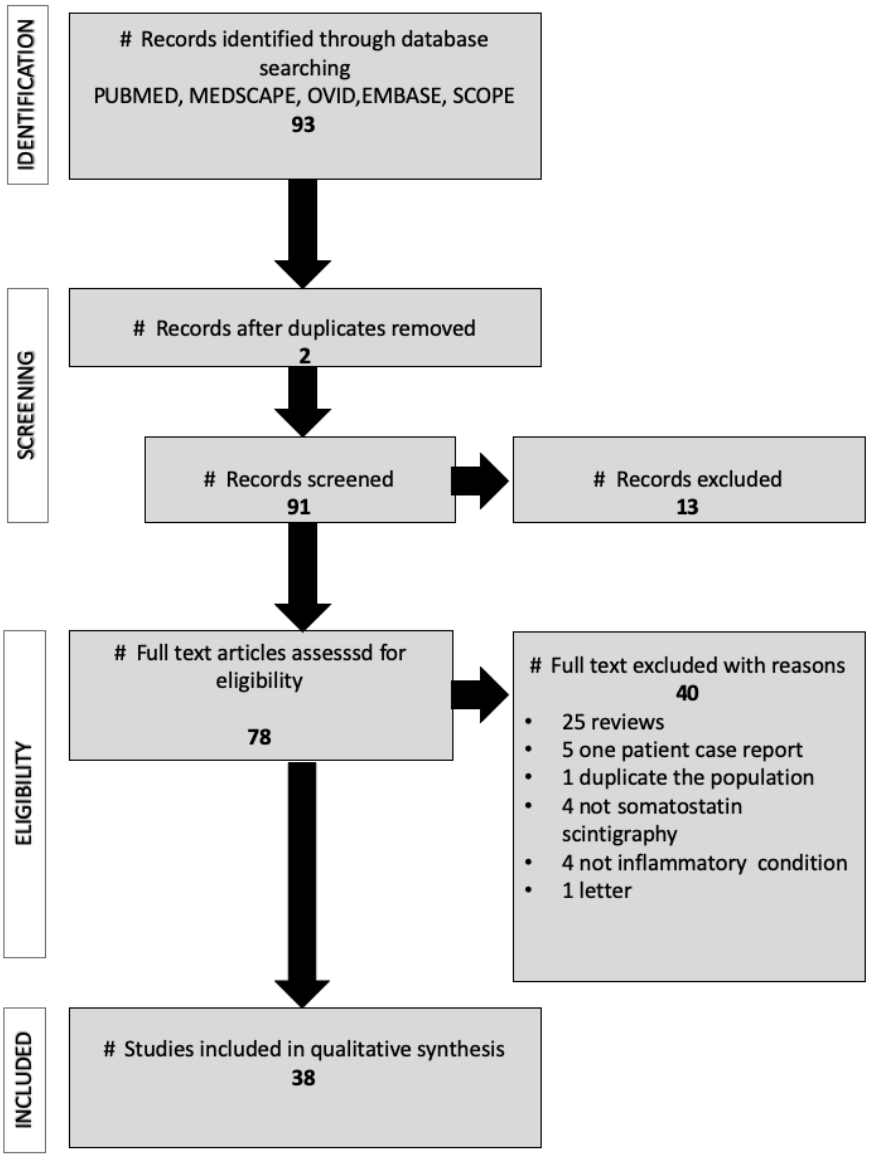

Figure 2

Graphical representation of frequencies of biases in analised papers by QUADAS 2.

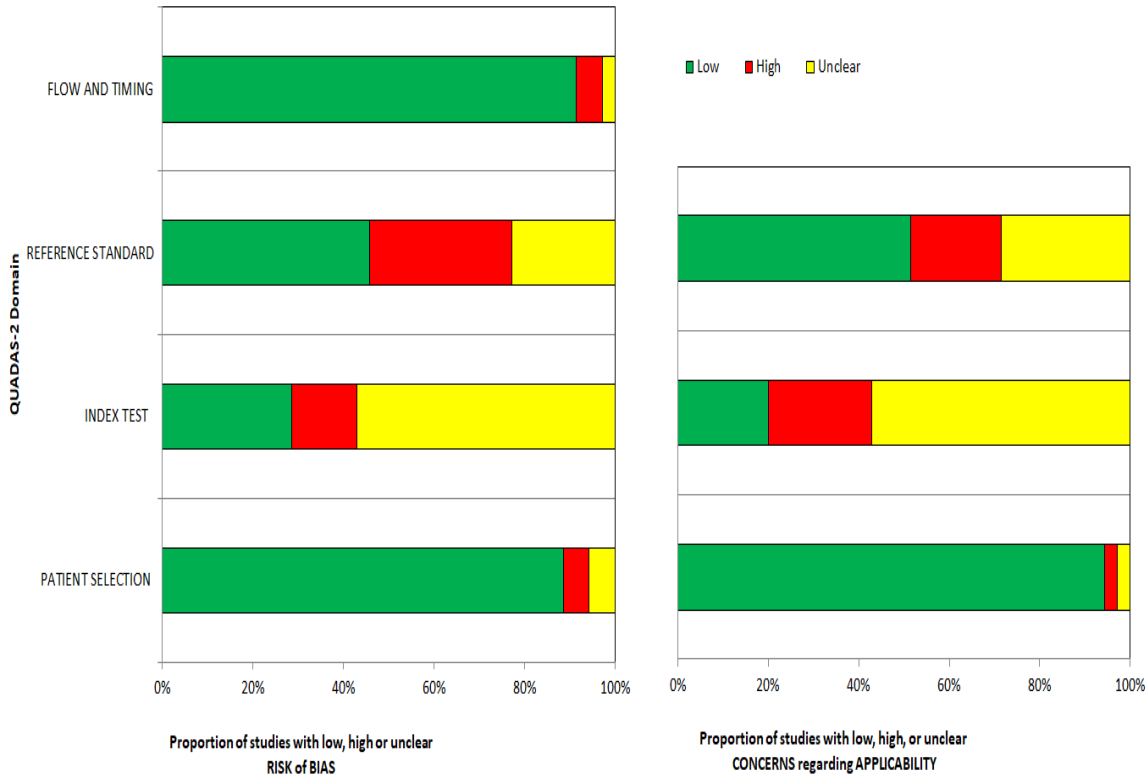

Figure 3
Graphical representation of biases in each analised papers by QUADAS 2.

Table 1 Summary of analyzed papers

Author	Type of study	Patients	Radiotracer	Risk of bias	Comments
Endothelial inflammation					
Tarkin 2001[18]	Prospective	42	^{18}F-FDG and ^{68}Ga-DOTA-TATE	We did not identify source of bias	SRS identifies culprit coronary and carotid arteries in individuals with acute coronary syndrome, TIA and stroke
Malmberg 2015 [19]	Observational prospective	60	^{64}Cu-DOTA-TATE	Reference test: they did not use reference test	SUVmax of inflamed plaques correlates with some Framingham risk factors
Wan 2017 [21]	Prospective cohort	20	^{68}Ga-DOTA-TATE	Flow and timing: Heterogeneity of time between carotid event, PET scan and endarterectomy	No significantly different uptake in carotid plaques and contralateral carotids and no inflammatory cells at histology of excised plaques
Pedersen 2015 [22]	Prospective cohort	10	^{64}Cu-DOTA-TATE	None	Uptake was significantly higher in symptomatic plaque versus the contralateral carotid artery and correlates with CD163 staining of plaques
Xian Li 2012 [23]	Descriptive retrospective series of cases	16	^{18}F-FDG and ^{68}Ga-DOTA-TATE	Reference standard and index test: unprecise reference test and lack of standard method for index test analysis.	Correlation between mean uptake of ^{18}F-FDG or ^{68}Ga-DOTA-TATE and patients' score of risk factors
Mojtahedi 2014 [24]	Retrospective series of cases	44	^{68}Ga-DOTA-TATE	Reference standard: Inaccurate method	^{68}Ga-DOTA-TATE detects more areas with increased uptake in patients with high cardiovascular risk
Rominger 2010 [25]	Descriptive, retrospective	70	^{68}Ga-DOTA-TATE	Reference standard: Inaccurate method.	^{68}Ga-DOTA-TATE detects more areas with increased focal uptake in patients with high cardiovascular risk and with calcified plaques
Rheumatoid arthritis					
Anzola 2016 [30]	Pilot study	18	^{99m}Tc-Hynic-TOC	Index test: Lack of standard method for index test analysis.	By ^{99m}Tc-Hynic-TOC all patients showed uptake in joints and in 60% of salivary glands. Patients who were evaluated after Infliximab therapy showed significant reduction of joint uptake.
Van Hagen 1994 [31]	Prospective	18	^{111}In-Octreotide	Index test and reference standard: just 2 patients were confirmed with histopathology and lack of standard method for index test analysis.	76% of swollen RA joints were visualized. The degree of pain and swelling correlated well with positive scintigraphy findings in joints
Cardiac allograft rejection					
Aparici 2000 [61]	Prospective	10	^{111}In-octreotide	Index test: lack of standard method for index test analysis.	Preliminary results indicate the feasibility of targeting activated lymphocytes with SRS in the detection of cardiac allograft rejection.
Small vessel vasculitis					
Neumann 2004 [62]	Pilot study	36	^{111}In-octreotide	Reference standard: lack of immunohistochemical analysis to confirm presence of SSTR2 and SSTR3 in the whole population.	^{111}In-octreotide for pulmonary disease showed a sensitivity of 86%, specificity of 96% and 97% of positive predictive value for active disease. For ear nose and throat disease 68% and 100%
Idiopathic pulmonary fibrosis					
Ambrosini 2010 [34]	Prospective	14	^{68}Ga-DOTA-NOC	Reference standard index test: it was not possible to confirm the presence of SSTR2	^{68}Ga-DOTA-NOC uptake corresponded to areas of HRCT abnormalities in IPF patients, supporting the hypothesis that SSTR is over- expressed in lungs of IPF patients.

Table 1 (continued)

Author	Type of study	Patients	Radiotracer	Risk of bias	Comments
				and SSTR3 in the affected areas. Lack of standard method for index test analysis.	
Lebtahi 2006 [35]	Prospective	11	^{111}In-octreotide	Index test and reference standard: Lack of standard method for index test analysis. Inaccurate reference test.	Increased uptake of ^{111}In-Octreotide in (mainly idiopathic) pulmonary fibrosis. Lung uptake correlates with alterations in lung function and with intensity of alveolitis and seems to be related to severity of lung fibrosis.
Win Thida 2012 [36]	Prospective	26	^{18}F-FDG and ^{68}Ga-DOTA-TATE	Patient selection, index test, reference standard: Patient heterogeneity of the population, few biopsies just in some of the patients; no threshold defined to interpret the index test.	All patients demonstrated increased pulmonary PET signal with ^{68}Ga-DOTA-TATE and ^{18}F-FDG. ^{68}Ga-DOTA-TATE might be useful to identify SSTR overexpression in this patient subgroup, and might be used for selecting patients who might benefit from somatostatin analog treatment.
Sarcoidosis and granulomatous diseases					
Lapa 2016 [51]	Prospective	15	^{68}Ga-DOTA-TOC	No source of bias.	Extent of ^{68} Ga DOTATOC PET positive myocardial areas might provide the same prognostic information as shown for ^{18}F-FDG-PET/CT in myocardial sarcoidosis. Inflammatory cells.
Kwekkeboom 1998 [52]	Cross-sectional	46	^{111}In-octreotide	Reference test and index test: not all patients had histological proof of granuloma. Lack of standard method for index test analysis.	^{111}In-Octreotide somatostatin receptor imaging can demonstrate active granulomatous disease in patients with sarcoidosis.
Lebtahi 2001 [53]	Prospective	18	^{111}In-octreotide and ^{67} gallium	Reference test and index test lack of in vitro analysis of somatostatin receptor presence in lesions. Lack of standard method for index test analysis. Some patients were receiving steroids previous to the scan.	^{111}In-octreotide SSTRs seems to be a promising alternative for evaluating the extent of sarcoidosis, detects significantly more sites of sarcoidosis involvement, especially for lung and mediastinal involvement.
Kamphuis 2015 [54]	Retrospective	175	^{111}In-Octreotide	Reference standard and index test: lack of in vitro analysis of somatostatin receptor expression in the lesions. Lack of standard method for index test analysis.	^{111}In-Octreotide SRS is additional in the diagnostic workup and more sensitive than conventional imaging in sarcoidosis patients.
Gormsen 2016 [55]	Pilot study	19	^{68}Ga-DOTA-NOC	Patient selection, reference standard, index test: 3 out of 19 patients had confirmed cardiac sarcoidosis; lack of a real reference test to diagnose the cardiac sarcoidosis; variation observer during the analysis of the index test.	^{68}Ga-DOTA-NOC can be used as an adjunct imaging modality in patients with suspected cardiac sarcoidosis; preferably as an imaging substitute for the obsolete ^{67}Ga-citrate scintigraphy.
Nobashi 2016 [56]	Retrospective	20	^{68}Ga-DOTA-TOC ^{67} Ga	Reference standard: lack of in vitro analysis of SSTR in the lesions.	^{68}Ga-DOTA-TOC was superior than ^{67}Ga-citrate identifying lymph nodes and in visualizing lesions in the uvea and muscle as well as in the lymph nodes.
Piotrowoski 2012 [57]	Observational	32	^{99m} Tc-HYNIC-TOC	Reference test and index test: reference test with limitations to detect extrapulmonary disease. Lack of standard method for index test analysis.	Although ^{99m}Tc-Hynic-TOC could discriminate between positive and negative studies further studies are needed to find the utility of those results.
	Pilot study	13	^{111}In-Octreotide		

Table 1 (continued)

Author	Type of study	Patients	Radiotracer	Risk of bias	Comments
Weinmmann 2000 [58]				Reference test and index test: not all patients went o biopsy to confirm the activated SSTRs; Lack of standard method for index test analysis.	^{111}In-Penteotride identified abnormal uptake in lungs, bone, but not in the skin nor in the liver and central nervous system in patients with granulomatous disease.
Oztürk 1994 [59]	Descriptive analysis	3	^{111}In-octreotide	Descriptive study on three cases.	Descriptive study showed increased uptake of ^{111}In-penteotride in granulomatous lesions.
Vanhagen 1994 [60]	Prospective	20	^{111}In-octreotide	Index test: Lack of standard method for index test analysis.	Descriptive study showed increased uptake of ^{111}In-Penteotride in granulomatous lesions
Thyroid exophthalmopathy					
Aguirre Balsalobre 2007 [37]	Series of cases	18	^{111}In-Octreotide	Index test: Lack of standard method for index test analysis.	^{111}In-Octreotide identified active thyroid orbitopathy. Patients identified as positive, treated with lanreotide showed improvement clinically and scintigraphically.
Colao 1998 [38]	Series of cases	10	^{111}In-Octreotide	Index test: Lack of standard method for index test analysis.	They showed that in a semiquantitative analysis with ^{111}In-pentreteotide for patients with higher scores in the orbital uptake, it is possible to predict the therapeutical outcome in these patients.
Burggasser 2003 [47]	Observational prospective	44	^{99m}TcP829	Index test: Lack of standard method for index test analysis.	Orbital uptake ratios were significantly different between patients with active and non-active orbitopathy. A statistically significant correlation was found between the CAS of the orbital disease and ^{99m}TcP829 tracer uptake.
Gerdin 1999 [39]	Prospective observational	22	^{111}In-Octreotide	Index test: Lack of standard method for index test analysis.	Quantitative measurement of orbital ^{111}Octreotide uptake might be of use in predicting the outcome of immunosuppressive and radiotherapy treatment of patients with Graves ophthalmopathy.
Huan Sun 2007 [48]	Prospective observational	14	^{99m}Tc-Hynic-TOC	Index test: Lack of standard method for index test analysis.	Orbital ^{99m}Tc-Hynic-TOC can be useful for the estimation of disease activity and prediction of the response to radiotherapy in Graves' ophthalmopathy patients
Kahaly 1998 [40]	Prospective observational	20	^{111}In-octreotide	Index test: lack of standard method for index test analysis.	^{111}In-octreotide was a sensitive technique with a high positive predictive value to select those patients who might benefit from treatment with immunosuppressive agents.
Kahaly 1995 [41]	Prospective observational	44	^{111}In-Octreotide	Index test: Lack of standard method for index test analysis.	Graves' ophthalmopathy patients showed markedly orbital accumulation of ^{111}In-pentetreotide in contrast to controls.
Krassas 1999 [42]	Prospective	14	^{111}In-Octreotide	Index test: Lack of standard method for index test analysis.	^{111}In-Octreoscan correlates well with the clinical activity of the thyroid exophthalmopathy and orbital accumulation of radioactivity was diminished after treatment with somatostatin analogues.
Krassas 1995 [43]	Prospective	20	^{111}In-Octreotide	Index test: Lack of standard method for index test analysis.	^{111}In-Octreoscan could predict those patients with Graves' thyroid eye disease who might benefit from treatment.
Lincke 2009 [50]	Prospective	73	^{111}In-pentetreotide and ^{68}Ga-DOTA-TOC	Index test, reference standard: lack of standard method for index test analysis; there was not immunohistochemical confirmation of activated SSTRs in the thyroid nodules.	Normal thyroid tissue shows detectable ^{111}In-Pentetreotide and ^{68}Ga-DOTA-TOC which could indicate a basal SSTR expression in normal tissue. Hot nodules showed increased uptake of both tracers also for active

Table 1 (continued)

Author	Type of study	Patients	Radiotracer	Risk of bias	Comments
					Hashimoto and Graves.
Moncayo 1999 [44]	Prospective	51	^{111}In-octreotide	Index test: lack of standard method for index test analysis.	Positive ^{111}In-Octreotide in patients Images provide useful information on the efficacy of immunosuppressive therapy.
Nocaudi 1999 [45]	Prospective	17	^{111}In-Octreotide	Index test: lack of standard method for index test analysis.	^{111}In-pentreteotide scintigraphy may be a good indicator of the likelihood of evolution in thyroid-associated ophthalmopathies.
Postema 1994 [46]	Cross sectional	58	^{111}In-Octreotide	Patient selection, index test: lack of standard method for index test analysis.	Thyroidal and orbital Graves' disease can be visualized by ^{111}In-Octreotide reflecting disease activity.
Rong Zhao 2012 [49]	Prospective	46	^{99m}Tc-Hynic-TOC	Index test: lack of standard method for index test analysis.	Orbital 99mTc-TOC fusion imaging is able to determine the pathological phase of Graves' disease, giving a high positive scan in the active early phase and a low positive or negative scan in the stable end phase of the disease.

Chapter 3

99mTc-labeled Rituximab for Imaging B Lymphocyte Infiltration in Inflammatory Autoimmune Disease Patients

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Abstract

Purpose: The rationale of the present study was to radiolabel rituximab with 99m-technetium and to image B lymphocytes infiltration in the affected tissues of patients with chronic inflammatory autoimmune diseases, in particular, the candidates to be treated with unlabelled rituximab, in order to provide a rationale for 'evidence-based' therapy.

Procedures: Rituximab was labelled with ^{99m}Tc via 2-ME reduction method. In vitro quality controls of ^{99m}Tc-rituximab included stability assay, cysteine challenge, SDS-PAGE, immuno- reactive fraction assay and competitive binding assay on CD20+ve Burkitt lymphoma-derived cells. For the human pilot study, 350–370 MBq (100 μg) of ^{99m}Tc-rituximab were injected in 20 patients with different chronic inflammatory autoimmune diseases. Whole body anteroposterior planar scintigraphic images were acquired 6 and 20 h p.i.

Results: Rituximab was labelled to a high labelling efficiency (998%) and specific activity (3515– 3700 MBq/mg) with retained biochemical integrity, stability and

biological activity. Scintigraphy with ^{99m}Tc-rituximab in patients showed a rapid and persistent spleen uptake, and the kidney appeared to be a prominent source for the excretion of radioactivity. Inflamed joints showed a variable degree of uptake at 6 h p.i. in patients with rheumatoid arthritis indicating patient variability; similarly, the salivary and lacrimal glands showed variable uptake in patients with Sjögren's syndrome, Behçet's disease and sarcoidosis. Inflammatory disease with particular characteristics showed specific uptake in inflammatory lesions, such as, dermatopolymyositis patients showed moderate to high skin uptake, a sarcoidosis patient showed moderate lung uptake, a Behçet's disease patient showed high oral mucosa uptake and a polychondritis patient showed moderate uptake in neck cartilages. In one patient with systemic lupus erythematosus, we did not find any non-physiological uptake.

Conclusion: Rituximab can be efficiently labelled with ^{99m}Tc with high labelling efficiency. The results suggest that this technique might be used to assess B lymphocyte infiltration in affected organs in patients with autoimmune diseases; this may provide a rationale for anti-CD20 therapies.

Key words: Rituximab, Anti-CD20 antibody, Radiolabelling, Molecular imaging, Therapy decision making.

Introduction

Rheumatoid arthritis (RA), psoriatic arthritis (PsA), Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), dermatopolymyositis, Behçet's disease, sarcoidosis and polychondritis are chronic inflammatory autoimmune diseases, and their treatment is often complicated. It has been shown that targeting B cells can directly alter autoimmune responses (1) in patients with these diseases. In the last few decades, our knowledge about the crucial role of B lymphocytes in disease pathogenesis has been increased from the advancement made in the under- standing of human immune system, including mechanisms of lymphocyte activation and antigen processing, immune tolerance, T and B lymphocytes crosstalk, and the role of pro-inflammatory cytokines in autoimmune processes (2-6). B cells are responsible for the production of auto-antibodies and rheumatoid factor and are also involved in T cell activation, pro-inflammatory cytokine production and there- fore play an important role in the pathogenesis of inflammatory autoimmune diseases (7,8). These cells have been found in pathological infiltrates in affected tissues of patients with autoimmune diseases and are implicated in disease progression (9).The development of mature B cells from stem cells involves several stages, each of which changes in expression of a wide range of cell surface markers. Thus, there are several potential candidates, on which B cell- depleting therapies can act directly, via use of monoclonal antibody (mAb) directed against cell surface markers (such as CD19, CD20, CD22) (10,11) or indirectly via blockade of cytokine pathways (such as TNF-α, interleukin-6, B lymphocyte stimulator (BLyS) and proliferation-inducing ligand APRIL) (12).CD20 is expressed on more than 95% of B lymphocyte from blood and lymphoid organs, therefore particularly suitable target for immunotherapy (7).This antigen expressed on the surface of B cell precursors, mature B cells and B cell lymphomas, but is not expressed on hemopoietic stem cells, pro-B cells, normal plasma cells, dendritic cells and other normal tissues. Anti-CD20 therapies include the humanized anti-CD20 mAbs ocrelizumab and veltuzumab, and the fully human mAb ofatumumab. These antibodies vary in the extent of

humanization and have different complement dependent cytotoxicity (CDC), and antibody-dependent cell-mediated cellular cytotoxicity (ADCC). Recently, TRU-015, a new anti-CD20 small modular immunopharmaceutical protein (SMIP) has been engineered, which is a dimeric, single-chain polypeptide (approximately one third to one half the size of mAb), for the treatment of RA and lymphoma (13,14).

Rituximab (MabThera®; F. Hoffmann-La Roche Ltd, Switzerland/Rituxan®; Biogen IDEC Pharmaceuticals Inc., USA), is an IgG1κ isotype chimeric anti-CD20 mAb that binds specifically to the transmembrane CD20 antigen. Rituximab was the first chimeric mAb approved by the United States Food and Drug Administration in 1997 for the treatment of malignancy, and in 2006 for the treatment of patients with active RA, who do not respond to one or more tumor necrosis factor (TNF) antagonist therapies. Rituximab promote B cell lysis by CDC, ADCC and induction of apoptosis.

The above data highlighted the opportunity to use the radiolabelled anti-CD20 mAb probe for in vivo imaging of CD20 positive B lymphocyte infiltration in inflammatory lesions. Such a probe would also allow non-invasive evaluation of disease extent and severity in patients affected by autoimmune diseases thus allowing better staging of the disease, since this might be difficult to assess by other conventional techniques (15).This approach, moreover, may allow to perform an 'evidence-based biological therapy with a view to assessing whether the antibody will localize in an inflammatory foci before using the same unlabelled anti-CD20 for therapy. Since, biological therapies are expensive and can be associated with severe side effects, scintigraphy with radiolabelled rituximab might prove particularly important for the selection of patients to be treated with unlabelled rituximab and may also be useful in patient follow-up for monitoring the efficacy of therapy.

Materials and Methods

Antibody

Rituximab (MabThera®) was provided by F. Hoffmann-La Roche Ltd., Switzerland.

Labelling of Rituximab with ^{99m}-Technetium

Rituximab was labelled with ^{99m}-technetium using a direct, 2- mercapthoethanol (2-ME) reduction method, as previously de- scribed (16). Briefly, disulfide bridges of the mAb were reduced by incubating a molar excess of 2-ME with rituximab solution (Mabthera®), for 30 min at room temperature in the dark. Different molar ratios between 2-ME: mAb (1,000:1, 2,000:1 and 4,000:1) were used in order to achieve the best activation of antibody and consequently the highest labelling efficiency (LE). Before labelling, activated antibody was purified by G-25 Sephadex PD10 desalting columns (GE Healthcare) and N_2 purged cold phosphate buffer saline (pH 7.4) as eluant. After activation and purification, the antibody was aliquoted in 100 μg each vial, and stored at −80°C, up to their use for radiolabelling.

Methylene diphosphonic acid (MDP) was used as weak trans- chelating ligand. The bone scan kit (Osteocis®, CIS Bio International) containing 3 mg methylene diphosphonic acid, 0.45 mg $SnCl_2.2H_2O$, 0.75 mg of ascorbic acid, 10.0 mg of sodium chloride was reconstituted with 1 ml of N_2 purged normal saline solution. Different amounts (from 1 to 10 μl) of methylene diposphonate solution were tested with 100 μg of activated antibody and 370 MBq of $^{99m}TcO_4^-$ freshly eluted from a $^{99}Mo/^{99m}Tc$ generator in order to achieve the highest LE. In the preparation of the radiopharmaceutical, all clinical grade reagents were used under sterile conditions.

Radiochemical Purity

Quality controls were performed using Instant Thin Layer Chromatography-Silica Gel (ITLC-SG) strips (VWR International). The strips were analyzed on a radioscanner (Bioscan Inc.) to quantitate the percentage of activity bound to the mAb. When 0.9% NaCl was used as the solvent (with normal ITLC-SG strips), retention factors (R*f*) were: ^{99m}Tc-rituximab=0.0; ^{99m}Tc-MDP and free $^{99m}TcO^-$=

0.9–1.0, whereas elution of albumin absorbed ITLC-SG strips with NH_3/H_2O/ethanol (1:5:2) resulted in R*f* values of: ^{99m}Tc-colloids= 0.0; ^{99m}Tc-rituximab, and free $^{99m}TcO_4^-$=0.9–1.0.

Stability

Stability of ^{99m}Tc-rituximab in human serum and normal saline was measured up to 22 hours, in four replicates. One milliliter of fresh human serum was added, in each of four aliquots of radiolabelled rituximab (100 µg) and incubated at 37°C. In another four aliquots of radiolabelled rituximab (100 µg), 1 ml of normal saline was added in each, was added and incubated at room temperature. The percentage of free $^{99m}TcO^-$ and radioactivity bound to mAb were measured at different time points (1, 3, 6 and 22 h) by ITLC-SG (as described above).

A cysteine challenge assay was also performed to check the *in vitro* stability of radiolabelled antibody at 37°C for 60 min, in four replicates. ^{99m}Tc-rituximab was incubated at different molar ratios of cysteine and mAb, ranging from 64:1 at the highest cysteine concentration to zero in the absence of cysteine. At the end of the incubation time, each reaction mixture was evaluated by ITLC-SG, as described above. All known chemical forms of ^{99m}Tc-cysteine have *Rf* values between 0.5 and 1.0, when normal saline is used as an eluent.

Structural Integrity

Possible modifications induced by 2-ME reduction procedure on rituximab were tested by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) in non-reducing conditions. Equal amount of protein (Native mAb, activated mAb and radiolabelled mAb) in each lane (25 µg) were subjected to 8% SDS-PAGE, at 45 mA constant voltage, along with protein molecular weight marker (11–250 kD, BioRad). After 2 h of electrophoresis, the gel was stained with Coomassie brilliant blue G250 (Sigma-Aldrich) for 60 min and washed thrice (15 min each) with a methanol/H_2O/ acetic acid (4.5:4.5:1) solution.

Autoradiography of ^{99m}Tc-rituximab was also performed to check the incorporation of radioactivity in mAb after radiolabelling. For this purpose, radiolabelled mAb (11.1 MBq) was loaded in a separate lane of SDS-PAGE. The gel was exposed to a Kodak® BioMax photographic plate (Sigma-Aldrich) for 20

min, and the photographic plate was developed subsequently by using Kodak® GBX developer/ replenisher (Sigma-Aldrich) and Kodak® GBX fixer/replenisher (Sigma-Aldrich) solutions, according to standard procedure.

Sterility

Radiolabelled mAb was sterilized by filtration through 0.22 μm Millipore GV syringe filter (low protein-binding filter) into a sterile vial, under the aseptic condition. Sterility test was performed on single patient doses from three different batches, by direct inoculation method using hemoculture vials, Plus+ Anarobic/F and Plus+Arobic/F (BD BACTEC^{TM}, BD Biosciences); these vials were incubated at 37°C for 7 days.

In vitro competitive binding assay

To test the in vitro binding ability of ^{99m}Tc-labelled rituximab with specific receptors, a competitive binding assay was performed on CD20 positive, Burkitt lymphoma-derived cell line, RAJI (17). The cells were maintained in an RPMI 1640 culture medium (Sigma- Aldrich) supplemented with 10% heat inactivated foetal calf serum (Gibco), 1% L-glutamine (Gibco), 1% antibiotics (penicillin and streptomycin, Gibco), at 37°C in a 5% CO_2 humidified incubator. Cell viability and cell count was determined by trypan blue assay using a hemocytometer.

For binding experiment, ^{99m}Tc-rituximab at increasing concentrations (from 0.05 to 50 nM) were incubated in triplicate with 4×10^5 cells alone or in the presence of 100 molar excess of the unlabelled antibody to saturate the specific receptor on cells. After 2 h of incubation, cells harvested by centrifugation (9,000×g for 3 min) and washed two times with complete RPMI 1640 culture medium. Cells and supernatants were collected in different vials and were counted separately for radioactivity in a single-well gamma counter (Gammatom s.p.a., Italy). The curve of specific binding was generated as difference between total binding and non specific binding. A Scatchard analysis was performed by using GraphPad Prism Version 5.00 Software (GraphPad Software, Inc.) to determine the dissociation constant (Kd).

Immunoreactive Fraction Assay

The assay for determination of the fraction of immunoreactive antibody by linear extrapolation to conditions representing infinite antigen excess has been adapted with slight modifications from the method described by Lindmo et al. [18].The immunoreactive fraction assay was performed using a constant concentration of radiolabelled mAb and serial dilutions of RAJI cells. The cells were washed three times in phosphate-buffered saline (pH 7.4) and suspended in a cold phosphate-buffered saline (PBS) with 1% bovine serum albumin (BSA) solution. Radiolabelled mAb at a constant concentration of 50 ng/ml, in PBS with 1% BSA solution was added to different amounts of cells (final concentration ranging from 2.6×10^6 to 0.08×10^6 cells/ml). Cells were incubated for 2 h at 4°C and then washed twice with 500 µl of cold PBS with 1% BSA solution, before counting cell-associated radioactivity in a single-well gamma counter. The data were plotted as a double inverse plot of the applied radiolabelled antibody over the specific binding as a function of the inverse cell concentration. In this plot, the origin of the abscissa represents infinite cell concentration, i.e., conditions of infinite antigen excess. All experiments were performed in duplicate.

Patients

We studied 20 consecutive patients (M/F, 4/16; mean age, 55.5± 11.65 years; mean disease duration, 12±99.3 months) who met with our inclusion criteria (stated below) and referred to the outpatient clinic of the rheumatology unit of 'Sapienza' University of Rome (Italy) due to chronic inflammatory autoimmune diseases, including RA, PsA, dermatopolymyositis, sarcoidosis, Sjögren's syndrome, SLE, Behçet's disease and polychondritis (n=5, 3, 5, 2, 2, 1, 1 and 1, respectively). All patients agreed to participate in the study and signed a written informed consent. None of the enrolled patient received unlabelled rituximab therapy before the study.

Inclusion criteria for patients were different depending on the type of pathology. RA: a moderate/high disease activity evaluated by a disease activity score (DAS) on 44 joints 93.7 (19), despite a treatment with disease modifying anti-

rheumatic drugs (DMARDs), including methotrexate at adequate dose and the failure of at least one TNF-α-blocker drug; all RA patients were candidates for a second level therapy as an anti-CD20 monoclonal antibody. PsA: a moderate/high disease activity evaluated by the swollen and tender joints count, patient and physician global assessment, despite a treatment with DMARDs and TNF-α blockers. Dermatopolymyositis: patients with an insufficient response to corticosteroids to commonly used immunosuppressants (azathioprine, mycophenolate, methotrexate or cyclosporine) or to high dose i.v. immunoglobulins, documented by clinical evaluation and altered laboratory tests. Sarcoidosis: a resistant disease to steroids, immunosuppressive drugs and/or antimalarial therapies. Sjögren's syndrome: an active disease, resistant to substitute agents for sicca features, glucocorticoids and immunosuppressive agents for extra glandular involvement. SLE: a moderate/severe disease activity evaluated by the systemic lupus erythematosus disease activity index (SLEDAI) (20), despite standard doses of steroid and immunosuppressive therapy. Behçet's disease: an active disease, resistant to standard treatment including immunosuppressive agents such as azathioprine and cyclosporine in combination with corticosteroids and colchicine. Polychondritis: a poorly controlled disease, despite nonsteroidal anti-inflammatory drugs, immunosuppressants (particularly methotrexate) or steroids.

Clinical assessment

Patients were evaluated by the same rheumatologist, and data were collected into a standardized case record forms, which included demographics, diagnosis, date of diagnosis, comorbidities, past and present treatments, biological therapies if prescribed, the start of the treatment and concomitant medications. Diagnosis of all mentioned diseases (RA, PsA, dermatopolymyositis, sarcoidosis, Sjögren's syndrome, SLE, Behçet's disease and polychondritis) were formulated according to international classification criteria for each illness (21-28).

Non-specific and specific laboratory tests, including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), muscle enzymes, immunological analysis

such as Anti-nuclear antibodies (ANA), extractable nuclear antigens (ENA) profile, rheumatoid factor, anti-citrullinated peptide antibodies and human leukocyte antigen (HLA)-B51 detection (only for Behçet's patient) were performed. When necessary, patients underwent other imaging procedures, such as chest radiography, computed tomography(CT) of chest, functional pulmonary tests and bronchoalveolar lavage (BAL) for sarcoidosis; osteoarticular ultrasonography (US) or magnetic resonance imaging (MRI) for RA and PsA; salivary glands US for Sjögren's syndrome; and muscles MRI for dermatopolymyositis.

Clinical evaluation in arthritis (RA and PsA) patients included count of swollen and tender joints, patient and physician global assessment (visual analog scale, VAS). Overall disease activity was also measured for RA patients with the DAS 44 which takes in consideration pain and swollen in 44 joints. A biopsy for tissues of interest, such as dermal, muscular and salivary gland was performed in dermatopolymyositis and Sjögren's syndrome patients.

Scintigraphic Studies with ^{99m}Tc-Rituximab

All patients underwent immunoscintigraphy, before treatment with unlabelled rituximab, to assess uptake of ^{99m}Tc-rituximab in the inflammatory lesions. Anterior planar whole body images were acquired 6 and 20 hours after i.v. administration of 350–370 MBq (100 μg) of ^{99m}Tc-rituximab. Planar anterior and posterior images of affected sites were also acquired at 6 and 20 hours. Images were acquired with a Philips Sky-Light dual head gamma camera fitted with a high resolution collimator. Whole body planar images were acquired on 256×1024 pixel matrix at 10 cm/min (at 6 h) or 5 cm/min (at 20 h) scanning speed. "Static regional images" were acquired on 512 x 512 pixel matrix for 300 seconds (at 6 h) or 600 seconds (at 20 h). A time- mode, rather than a count-mode acquisition modality was chosen to be able to compare images of different patients, being injected with almost the same dose of radioactivity, thus avoiding operator bias in image acquisition and analysis.

The scintigraphic scans were interpreted by two experienced nuclear medicine physicians. The interpreters had clinical information obtained from the patient's referring physician but no information on the other imaging modality. Nevertheless, the uptake interpretation were describe as mild, moderate and high comparing with the heart and large blood vessel activity. An uptake was considered as 'mild' when it was lower than heart and large blood vessel uptake, 'moderate' when it was equal to heart and large blood vessel uptake, and 'high' when it was higher than heart and large blood vessel uptake.

Statistical analysis

The student t-test was used to compare quantitative variables in the same group. A *p* value less than 0.05 was considered statistically significant.

Results

Radiochemical Purity

By using a direct labelling method, the best results were obtained when disulfide bridges of the antibody were reduced using a 2,000-fold excess of 2-ME. An increase in 2-ME concentration for reduction of rituximab, results in decreased LE, and higher colloid percentage. We obtained a high LE (998%) with negligible amount of colloids (G2%) with high specific activity (3,515–3,700 MBq/mg), when the activated mAb was labelled with ^{99m}Tc by using only 3 μl of methylene diphosphonic acid solution (from the bone scan kit). Thus, a post labelling purification step could be avoided.

Stability

Radiolabelled rituximab was stable when incubated in fresh human serum or in normal saline up to 22 h, as shown in Fig. 1a. After 22 h, still more than 80% of the radioactivity was bound to the antibody in both media. The results of cysteine challenge assay also demonstrated that radiolabelled rituximab was stable, approximately 95% intact when exposed to up to a 16-fold excess of cysteine (Fig. 1b).

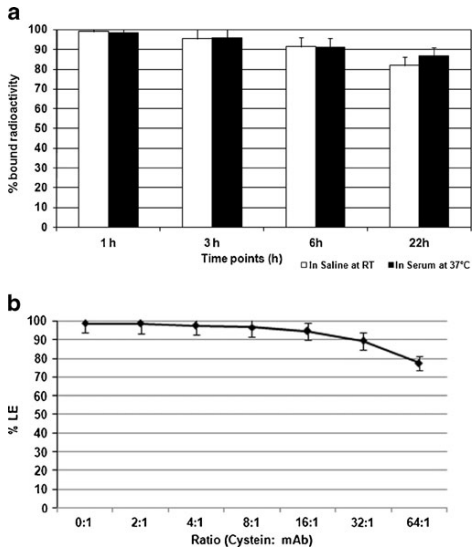

Fig. 1. a. Stability of ^{99m}Tc-rituximab in saline and in plasma assessed by ITLC-SG at different time points. b Cysteine challenge assay of ^{99m}Tc-rituximab assessed by ITLC-SG at increasing ratio between cysteine to mAb.

Structural Integrity

SDS-PAGE analysis of native, activated and radiolabelled rituximab show, one thick band of 145 kilodalton (kDa) in native rituximab lane; bands of different molecular weights in activated rituximab lane probably due to light/heavy chain complexes after 2-ME reduction; whereas, mainly one thick band of approximately 145 kDa in radiolabelled rituximab lane, i.e., the molecular weight of intact mAb (Fig. 2). Autoradiography of ^{99m}Tc-rituximab lane, showed that almost all radioactivity was attached with the band of higher molecular weight (145 kDa)

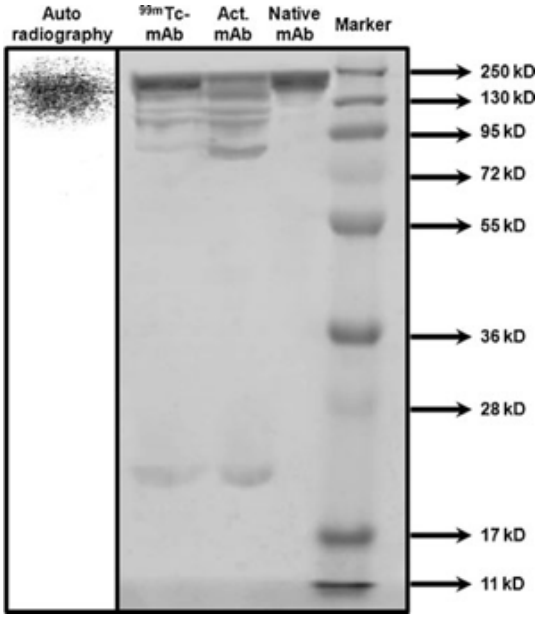

Fig. 2. SDS-PAGE analysis performed in non-reducing condition showing native, activated and ^{99m}Tc labelled rituximab lane. The autoradiography analysis (*first lane*) showing radioactivity associated with the band of complete mAb (145 kDa).

Sterility

The sterility test did not show any Gram+ve or –ve bacterial growth in hemoculture vials inoculated with ^{99m}Tc labelled rituximab.

In vitro competitive binding assay

The saturation binding curve was plotted as a specific bound radioactivity against increasing molar concentration of radiolabelled mAb showed a plateau (Fig. 3). A 100-fold molar excess of unlabelled antibody saturates the receptors present on cells, and consequently prevented the specific binding of the radiolabelled rituximab, which shows that rituximab retained its specific binding activity with CD20 receptors expressed on RAJI cells, even after the radio- labelling with technetium-99 m. Kd for ^{99m}Tc-rituximab 8.3 nM, which is only slightly higher than the Kd of native rituximab, i.e., 5.2 nM.

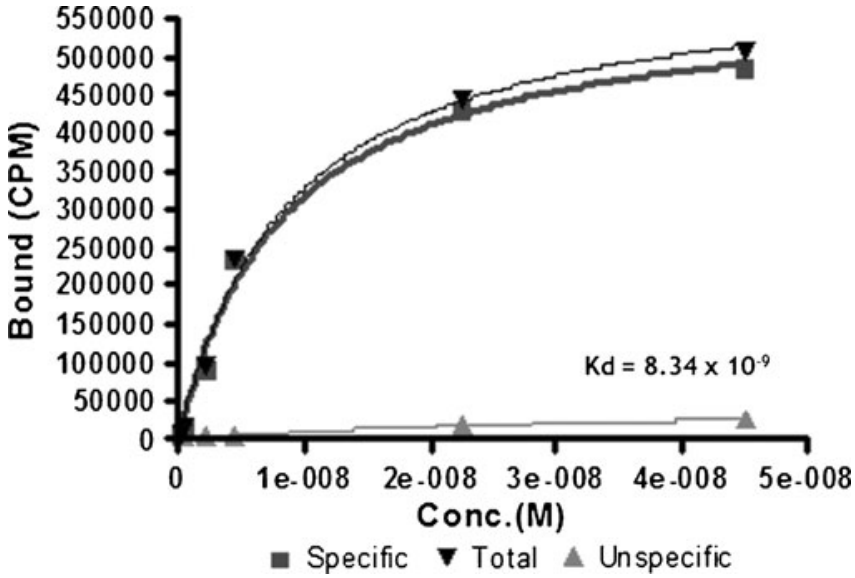

Fig. 3. Saturation binding curve of ^{99m}Tc-labelled rituximab to RAJI cells. Kd for ^{99m}Tc-rituximab 8.3 nM. Curve fitting was performed using GraphPad software.

Immunoreactive Fraction Ass*ay*

The data demonstrate a very close linear relationship of 'total applied/ specific binding' as a function of the inverse cell concentration. Fitting of a straight line to the data by means of linear regression analysis allows an easy and precise determination of the intercept value at the ordinate. This value equals 1/immunoreactive fraction; thus in this case, the immunoreactive fraction was 85.5%, as indicated in the Fig.4

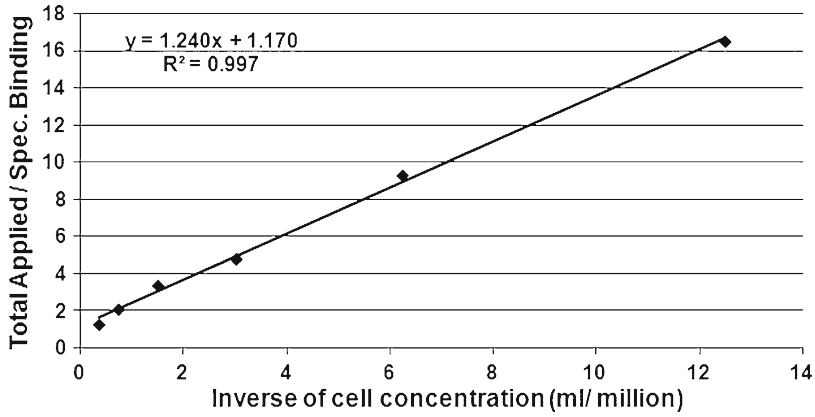

Fig. 4. A double inverse plot of the immunoreactivity fraction assay was used to determine immunoreactive fraction (i.e., 85.5%).

Clinical Assessment

In clinical evaluation, all RA patients had moderate pain, and swelling and/or tenderness in knees and wrists, ankles, shoulders. VAS was 75± 11.2, ESR was 29±22, CRP was 0.55±13.4, DAS 44 was 4.9±1.1. PsA patients had a mean ESR of 35±31 and CRP of 0.8±2.2; they also showed moderate pain and swelling and/or tenderness in the knees, wrists, ankles and shoulders. The ESR (millimeters per hour) and CRP (milligrams per liter) values, evaluated before the scan of patients with dermatopolymyositis, Sjögren's syndrome, sarcoidosis, SLE, Behçet's disease and polychondritis are shown in Table 1. Additionally, patients with dermatopolymyositis and Sjögren's syndrome had positive biopsies, and both Sjögren's syndrome patients were reported positive for ultrasonography (US) of the salivary glands. Moreover, both sarcoidosis patients had a positive BAL, and an oral aphthosis was noticed in Behçet's disease patient confirming the disease.

Table 1

Table 1. Clinical and scintigraphic findings of patients												
Diseases	№ of Patients	Sex	Age (yeas)	Disease Duration	99mTc-anti-CD20 mAb scintigraphy uptake at 6 h							
					Affected Joints	Lung	Salivary glands	Lacrimal glands	Skin	Cartilages	Buccal mucosa	Spleen
Rheumatoid arthritis	5	5 F	58±14.9	12.6±14.6	+/+++	−/+	-	-	-	+/+++	-	+++
VAS (mean±SD): 75 ±11.2 DAS 44 (mean±SD): 4.9±1.1		ESR (mm/h) (mean±SD): 29 ±22 Swelling: knees, ankles, shoulders, wrists							Tenderness: knees, ankles, shoulders, wrists	CRP (mean±SD): 0.55±13.4		
Psoriatic arthritis	3	1M; 2F	45.7±12.5	2.33±3.2	+/+++	−/+	-	-	-	+/+++	-	+++
ESR (mm/h) (mean±SD): 35±31 Tenderness: knees, ankles, shoulders, wrists		CRP (mean±SD): 0.8±2.2							Swelling: knees, ankles, shoulders, wrists			
Dermatopolymyositis	5	1M; 4F	58.8±5.9	5.4 ±5.6	−	−	-	-	+/+++	-	-	+/+++
ESR (mm/h) (mean±SD): 29±18		CRP (mean±SD): 0.5±0.1							Dermal biopsy: +ve			
Sjögren syndrome	2	2F	80, 66	1, 0.5	−/++	−/+	+/+++	++	-	-	−/+	+++
US: +ve salivary glands Salivary gland biopsy: +ve		ESR (mm/h) (mean±SD): 17, 19										
Sarcoidosis	2	1M; 1F	48, 58	1, 1	−/+	+/++	++/+++	+/++	−	-	-	+++
ESR (mm/h): 34, 19		CRP (mean±SD): 0.4, 0.25							BAL: +ve			
Systemic lupus erythematosus	1	F		0.5	−	+	-	-	-	-	-	+++
ESR (mm/h): 69		CRP: 0.44										
Behçet's disease	1	M		1	+	-	+++	++	-	-	+++	+++
ESR (mm/h): 3		CRP: 0.4							Swelling: two ankles			
Tenderness: left ankle, lumbalgia		Oral aphtosis										
Polychondritis ESR (mm/h): 15	1	F CRP:0 .1	54	3	-	-	+	-	-	++	-	+++

VAS visual analogue scale, ESR erythrocyte sedimentation rate, CRP C-reactive protein, DAS 44 disease activity score, US score ultrasonography score. (+, mild uptake; ++, moderate uptake; +++, high uptake; −, no detectable uptake of the radiopharmaceutical over background).

Scintigraphic Studies with ^{99m}Tc-Rituximab

After i.v. administration of ^{99m}Tc-rituximab (350–370 MBq; 100 μg), the whole body images were acquired at 6 and 20 h. We observed a mild to moderate to high uptake in the affected regions at 6 h p.i., in all the patients with different autoimmune diseases (Fig. 5a–e and Table 1). At 20 h, we found a low background activity and consequently higher target to background (T/B) ratio. In the five patients with active RA and the three patients with PsA, we observed an uptake of the radiopharmaceutical in the known affected joints (including knees, wrists, elbows, phalanges, ankles and shoulders). This uptake was

variable and not every patient did show uptake in each clinically affected joints (Fig.6a).Muscle and skin uptake in patients with dermatopolymyositis were detected; however, this kind of scintigraphy was more positive at 20 h when the background blood pool activity was decreased. Interestingly, in one dermatopolymyositis patient we found a very mild spleen uptake, in contrast to the high uptake normally present in all other subjects. After that, FACS analysis of peripheral blood cells confirmed the presence of a significantly low B lymphocyte count in this patient. Patients suffering from Sjögren's disease showed a variable ^{99m}Tc-rituximab uptake in salivary and moderate uptake lachrymal glands (Fig. 6b). Scintigraphy in the patient with Behçet's disease demonstrated clearly detectable buccal ^{99m}Tc-rituximab activity at 6 h p.i. (Fig.6c). We also performed scintigraphic examination in one patient with sarcoidosis in which a mild ^{99m}Tc-rituximab uptake was found in the nasal cavity, salivary and lachrymal glands (Fig. 6d) similar to the so called "Panda Sign" described for scintigraphy with ^{67}Ga-citrate (29). However, we could not see a high uptake in the lung region, as per our expectation. An SLE patient was difficult to evaluate. We found a mild radiopharmaceutical uptake in the lung region. This patient did not show uptake in clinically defined inflammatory lesions. The patient with polychondritis showed an abnormal radiopharmaceutical uptake in the cartilages of the neck (thyroid and cricoid cartilages) and in a regional lymph node. Some faint uptake was also detectable in the sub- mandibular salivary glands.

A variable thyroid uptake was detected in ten out of 20 patients (50%). We therefore wanted to investigate whether there was an autoimmune reaction in these patients and we asked them to test anti-thyroglobulin (anti-Tg) and anti-thyroid peroxidase antibody (anti-TPO). Interestingly, five of these patients showed increased anti-thyroid auto-anti- bodies, whereas amongst the ten patients without thyroid uptake of ^{99m}Tc-rituximab, none had increased anti-thyroid auto-antibodies.

None of the patients showed any kind of side effect, adverse event or other type of reaction following the administration of the radiotracer used, either immediately after injection or after 1 month.

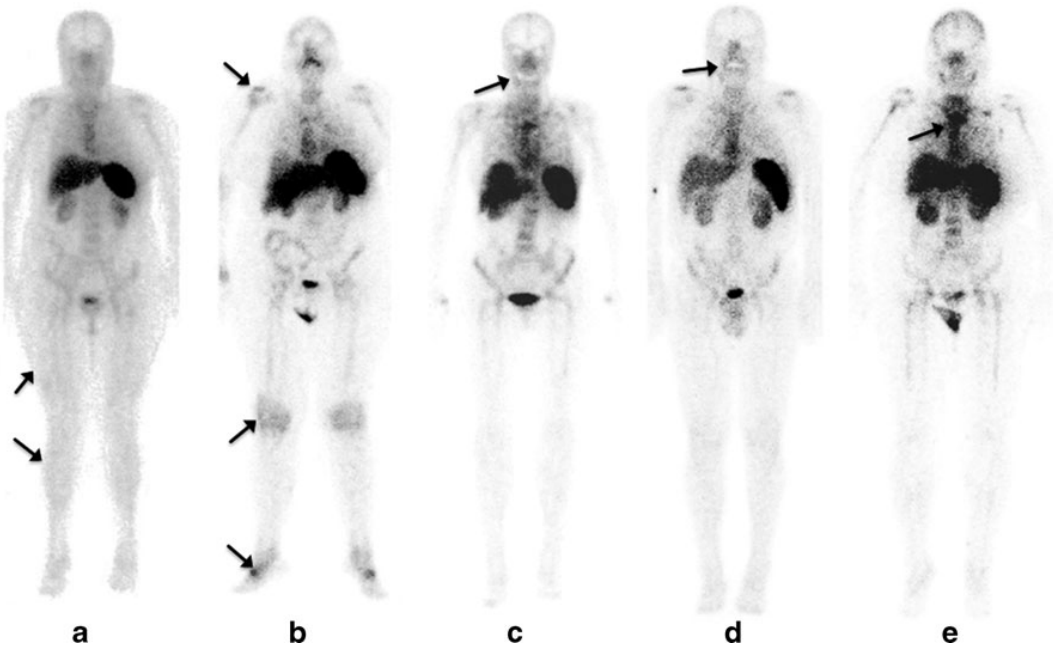

Fig. 5. Whole body scintigraphic images at 6 h with ^{99m}Tc-rituximab in patients with a dermatopolymyositis (see skin uptake). b Rheumatoid arthritis (see joint uptake). c Sjögren's syndrome (see salivary and lacrimal gland uptake). d Behçet's disease (see oral mucosa uptake). e Sarcoidosis (see lung uptake).

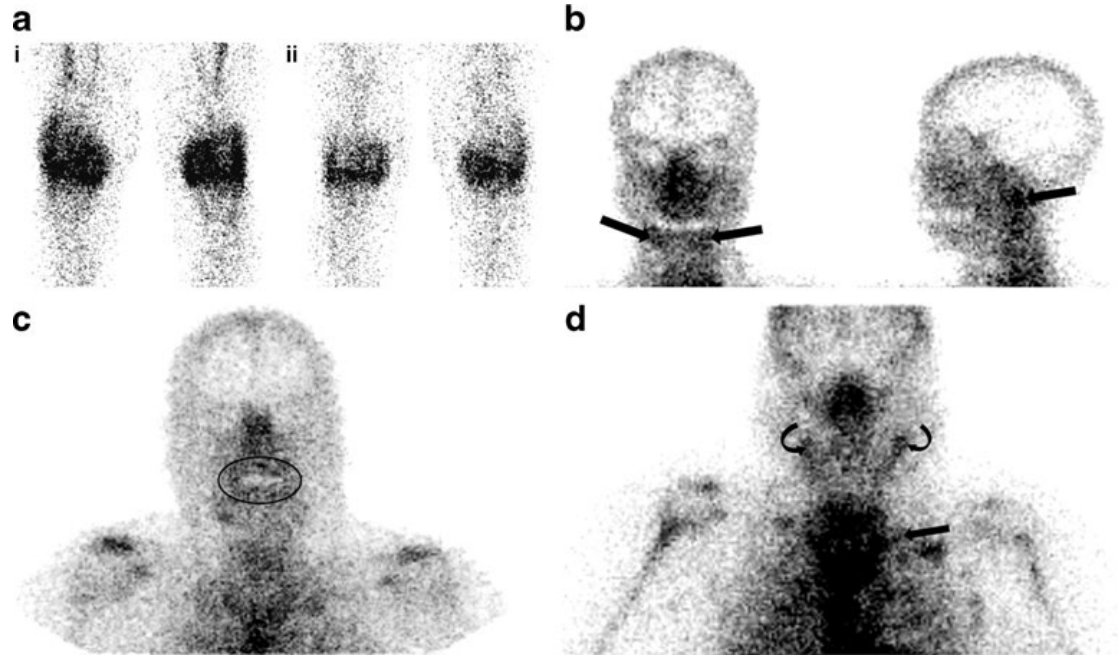

Fig. 6. Static regional scintigraphic images with ^{99m}Tc-rituximab in patients with a Rheumatoid arthritis (at *i* 6 h and *ii* 20 h). b Sjögren's syndrome (at 6 h). c Behçet's disease (at 6 h). d Sarcoidosis (at 6 h).

Discussion

Previous studies with radiolabelled non-specific probes (such as ^{99m}Tc-human polyclonal immunoglobulins, ^{67}Ga- citrate, ^{99m}Tc-albumin nanocolloids, ^{18}F-FDG, ^{99m}Tc- and ^{111}In- labelled liposomes) were focused on the detection of the state of activity of the disease (30,31). Nevertheless, no real clinical advantages have been proved comparing this radiopharmaceuticals to other available diagnostic techniques (such as ultrasonography, X-ray, magnetic resonance imaging) with regard to the clinical management of patients. The real breakthrough in targeted immunoscintigraphy for inflammatory disease patients is the possibility to highlight the presence of the relevant receptors involved in the pathophysiology of the disease directly by means of the specific radiolabelled mAbs that will eventually be used for treatment. Some radiolabelled mAbs (such as anti-E-selectin and anti-CD4) demonstrated their excellent capability for the localization of inflammatory regions, but lack of their

use for the therapeutic purposes, thus limiting their further development and use for immunoscintigraphy. However, approval of mAbs for therapeutic purposes (such as anti-CD20 for treatment of rheumatic patients) provides us an opportunity to select the patients through this technique. In the present study, we aimed to radiolabel rituximab with ^{99m}Tc for scintigraphic studies, and then, as a proof of concept, we evaluated its in vivo localization pattern in a cohort of patients affected by different autoimmune diseases. The results obtained so far are highly encouraging and hold promise for therapy decision making and follow-up, with a view to assessing whether an antibody will accumulate in an inflamed tissue before using the same unlabelled antibody for therapeutic purposes. This kind of information can only be obtained with such kind of imaging approach based on new radiopharmaceuticals that provide a solid basis for the further development and clinical use of immunoscintigraphy in inflammatory autoimmune disease patients.

We demonstrated that rituximab could be labelled with ^{99m}Tc without any modification in its biological activity and specificity for CD20 receptors *in vivo*. Direct radiolabelling method using 2-ME reduction of antibody, is simple, rapid, reliable and yielding high LE with excellent in vivo targeting. We always labelled antibody to a high labelling efficiency (998%) and specific activity (3,515–3,700 MBq/mg). SDS-PAGE and autoradiography analysis demonstrated that the radioactivity stands with whole antibody molecule (band of 145 kD). Our immune reactive fraction assay demonstrated that 85.5% of the antibody was immune reactive even after the radiolabelling procedure.

For scintigraphic studies, we injected a tracer dose of 350–370 MBq (100 μg) of rituximab, which is a much lower dose in comparison with therapeutic dose (with no adverse or allergic reaction) that is unlikely to alter the natural history of the disease or have any therapeutic effect. In patients, at 6-h images, ^{99m}Tc-rituximab was detectable in the heart and large vessels, liver, spleen and kidneys with more variable uptake in the lungs, thyroid and bone marrow. Rapid and persistent uptake of the spleen was also concordant with results recently published by Stopar et al. in patients with B cell non-Hodgkin's

lymphoma (32). At 20-h images, we observed reduction of blood pool activity and lung activity with increased liver and spleen activity. Bowel activity was never detected. Thyroid and salivary gland uptake was not always concordant, and stomach uptake was never detected. Thyroid uptake could be correlated to the presence of subclinical autoimmune phenomena in this organ, since the presence of anti-thyroid auto-antibodies in five out of ten patients with thyroid uptake. In the remaining five patients (with thyroid uptake but without anti-thyroid auto-antibodies) we cannot exclude the presence of a "silent" lymphocyte infiltration as well as possible uptake of free Tc, the latter possibility being, however, unlikely because of the absence of salivary gland uptake in these patients. The use of radiolabelled mAbs against lymphocyte antigens for therapy decision making was already explored by us with success and clinical benefit. The most clinically relevant example is the immunoscintigraphy with ^{99m}Tc- anti-TNF-α mAb in Crohn's disease (CD) and RA patients (16,33). In the first study in CD patients, ^{99m}Tc-infliximab (a commercially available mAb anti-TNF-α) showed uptake in the affected bowel only in few patients that responded to anti-TNF treatment (16). In another study in patients with active RA before intra-articular treatment with infliximab, we observed a variable degree of joint uptake that did not correlate with swelling or pain of the joint, but could predict the success of therapy (33). These studies demonstrated that radiolabelled anti-TNF-α scintigraphy could be useful for improving the selection of those patients who could benefit most from therapy with TNF-α antagonists, an example of the use of immunoscintigraphy for therapy decision making. In line with this strategy, in the present study, scintigraphic images with ^{99m}Tc-rituximab demonstrated moderate to high accumulation in some affected joints of arthritis patients, but some other swelling and painful joints were negative at the scan. This suggests that not all joints have a similar pattern of B lymphocyte infiltration and, therefore, the total-body evaluation of CD20 positivity should be mandatory before initiating anti-CD20 therapy. Overall, our data demonstrated different biodistribution of radiolabelled CD20 in different patients, thus different degrees of B lymphocyte infiltration in

different tissues of different patients. Our results are encouraging and hold promise not only for imaging of B lymphocyte infiltration in inflammatory diseases but, most importantly, for mapping B lymphocytes in affected tissues of each patient in order to establish a personalized therapy. Despite large prospective studies being required to confirm the clinical utility of this technique in each disease, data obtained so far indicate that the information about the presence of drug targets for personalized therapy, by means of immunoscintigrapy, is of great clinical and social relevance for optimizing treatments, avoiding unnecessary therapies and reducing costs thus providing a cost-effective solution for biological therapies.

Conflict of Interest Disclosure. The authors declare that they have no conflict of interest.

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Chapter 4

Somatostatin receptor scintigraphy in patients with rheumatoid arthritis and secondary Sjögren's syndrome treated with Infliximab: a pilot study.

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Abstract

Background: Human T lymphocytes infiltrating tissues in autoimmune diseases are known to express somatostatin receptors amongst other activation markers. In this study, we evaluated whether somatostatin receptor scintigraphy (SRS) using a radiolabelled somatostatin analogue (^{99m}Tc-EDDA/tricine-HYNIC-tyr(3)-octreotide (^{99m}Tc-EDDA/HYNIC-TOC)) is able to detect the presence of immune-mediated processes in patients with rheumatoid arthritis and secondary Sjögren's syndrome. We also aimed to evaluate whether positivity to SRS was predictive of therapeutic response and if SRS could be used for monitoring the efficacy of immunomodulatory treatment.

Methods: Eighteen patients with rheumatoid arthritis and secondary Sjögren's syndrome not responding to conventional treatment were recruited for treatment with infliximab, a monoclonal antibody against TNF-α. All patients had complete blood cell count, renal and liver function tests, measurements of ESR, CRP, ANA, ENA, and anti-dsDNA antibodies, functional salivary gland scintigraphy, labial biopsy, and ophthalmologic assessment with Schirmer's test and tear film break-up time (BUT). Diagnosis was made according to the revised criteria of the American-European Consensus Group. All patients underwent SRS at baseline and after 3–6 months of therapy with infliximab. Eleven out of 18 had repeat SRS images. Images of the salivary glands and major joints were acquired 3 h after injection of 370 MBq of ^{99m}Tc-EDDA/HYNIC-TOC. Image analysis was performed semi-quantitatively.

Results: All patients showed uptake of ^{99m}Tc-EDDA/HYNIC-TOC in the joints. Salivary glands also showed variable radiopharmaceutical uptake in 12 out of 18 patients, but all patients showed presence of lymphocytic infiltration at labial salivary gland biopsy. All patients, who repeated the study after treatment, showed significant reduction of somatostatin uptake in the joints but not in the salivary glands.

Conclusions: SRS using ^{99m}Tc-EDDA/HYNIC-TOC may be a useful imaging tool to assess disease activity and extent in patients with rheumatoid arthritis and may help to detect secondary Sjögren's syndrome. It may also aid therapy decision-making with anti-TNFα antibodies in the joints but not in salivary glands.

Keywords: Rheumatoid arthritis, Sjögren, Inflammation imaging, ^{99m}Tc-EDDA/HYNIC-TOC, Somatostatin receptor scintigraphy, Infliximab

Introduction

Sjögren's syndrome (SS) and rheumatoid arthritis (RA) are chronic inflammatory autoimmune diseases that may frequently coexist in affected patients. The former is characterized by a decrease in lacrimal and salivary secretion. It can be primary (idiopathic) or secondary (when associated with RA, ankylosing spondylitis, systemic lupus erythematosus, and others). SS is particularly relevant amongst autoimmune diseases because of its high incidence and unknown aetiology. In particular, secondary SS may be present in up to 30 % of patients with systemic lupus erythematosus and up to 20 % of patients with RA (1). The majority of affected patients are females (90 %), aged between 40 and 60 years. This might be related to the immunoregulatory properties of the sex hormones; however, some genetic and environmental factors may also play a role (2).The causes of SS have not been elucidated yet, but it is always characterized by a lymphocytic infiltration in the exocrine glands (mainly the salivary and lacrimal glands) and the presence of circulating autoantibodies that advocate for autoimmune phenomena. Cytokines derived from both T and B lymphocytes contribute to the destruction of glandular tissue and inflammation (3).

An important mediator of chronic inflammation in both RA and SS is the tumor necrosis factor alpha (TNF-α) which is a cytokine with stimulating or inhibiting activity directly on immune cells. Impairment of TNF-α production causes pro-inflammatory effects through the production of many cytokines, such as interleukin-8 (4). Given its role in autoimmune disorders, therapeutic approaches based on its blockage has been proposed. In particular, anti-TNF-α monoclonal antibodies (mAbs) such as infliximab or adalimumab have been used in patients affected by RA with positive results. Nowadays, infliximab is approved for the treatment of moderate to severe active RA, Crohn's disease, ulcerative colitis, ankylosing spondylitis, psoriatic arthritis,

and plaque psoriasis and is also pre- scribed (off label) for the treatment of Behçet's disease and sarcoidosis (5). However, its use in SS showed controversial results and recent trials in patients have failed to confirm any benefit of this therapy (6). Similarly, it has been reported that not all RA patients respond to therapy with anti-TNF-α antibodies(7). This led to the hypothesis that not all inflammatory processes in patients affected by RA and SS are mediated by TNF-α. Therefore, non-invasive tools to evaluate its presence in inflamed lesions would help clinicians in selecting patients who could benefit from infliximab therapy (8).

Over the last few years, somatostatin receptor scintigraphy (SRS) using somatostatin analogues has been widely used in diagnosing different types of inflammatory diseases, such as Graves' ophthalmopathy, granulomatous diseases, and rejection of cardiac allografts and in the formation of vulnerable atherosclerotic plaques (9). Indeed, somatostatin has regulatory effects on immune cells, associated with T cell function, and inhibits the production of cytokines such as TNF-α, IL-1, and IL-6 (10). Therefore, since somatostatin receptor type 2 is overexpressed by activated lymphocytes in chronic immune-mediated diseases (11), it could be a potential target for peptide receptor imaging.

The aim of this study was to evaluate whether SRS is capable of detecting the presence of immune-mediated processes in patients with RA and secondary SS before and after immunomodulatory therapy with infliximab. In addition, we investigated the effect of treatment on the function of salivary glands.

Materials and Methods

Patients

Scintigraphy with ^{99m}Tc-EDDA/tricine-HYNIC-tyr(3)-octreotide (^{99m}Tc-EDDA/HYNIC-TOC) was performed in 18 patients (2 males and 16 females; age range 18–70 years; mean age 46.5 ± 12.2 years) affected by both RA

and secondary SS, resistant to conventional treatment and diagnosed according to the revised criteria of the American-European Consensus Group for SS (12). In all patients, complete blood cell count, renal and liver function tests, erythrocyte sedimentation rate (ESR), C- reactive protein (CRP), antinuclear antibodies (ANA), extractable nuclear antigens (ENA), anti-dsDNA anti- bodies, labial biopsy, and ophthalmologic assessment with Schirmer's test and tear film break-up time (BUT) were performed. At the time of the study, all patients were receiving immunomodulatory drugs (cortisone and/or cyclosporine A and/or metrothexate).Three to 6 months after the end of the treatment with infliximab (protocol used in the ATTRACT study) (13), the SRS was repeated in 11 patients to evaluate the effect of the therapy on the inflammatory process in affected joints and salivary glands. Salivary gland scintigraphy (SGS) was also performed to evaluate the functional status of salivary glands pre and post treatment. In addition, 20 patients with neuroendocrine tumors (NETs), but without inflammatory lesions in joints and salivary glands, were included for ^{99m}Tc-EDDA/HYNIC-TOC scan to investigate the uptake in those sites. The study was approved by the Clinica Reina Sofia, Bogotà and Regina Apostolorum Hospital, Rome and was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Radiopharmaceutical

^{99m}Tc-EDDA/HYNIC-TOC, a somatostatin analogue labelled with ^{99m}Tc was used for SRS. This radiopharmaceutical binds with high affinity to type 2,3, and 5 somatostatin receptors (14) and was prepared from a commercially available kit (^{99m}Tc-Tektrotyd, POLA- TOM, Otwock, Poland), according to the manufacturer's instructions. Briefly, 740 MBq of ^{99m}Tc-pertechnetate in0.9 % NaCl solution (pH = 7) was added to a vial containing 20 µg HYNIC-Tyr^3-octreotide, 40 µg stannous chloride(II), 50 mg tricine, 10 mg mannitol, and 10 mg ethylenediaminodiacetic acid (EDDA). The solution was gently stirred and incubated at 80 °C for 30 minutes.

Scintigraphic imaging

Static planar images of all major joints and of the salivary glands were acquired 3 h after the i.v. injection ^{99m}Tc-EDDA/HYNIC-TOC (370 MBq) for 10 min using a 512 × 512 matrix. Thirty minutes before the radiopharmaceutical injection, patients were treated with 400 mg of $KClO_4$ (Pertiroid®) to prevent the uptake of free $^{99m}TcO^-$ possibly released by the catabolism of ^{99m}Tc-EDDA/HYNIC-TOC.

Functional sialoscintigraphy (SGS) was performed on a separate day and less than 5 days after SRS, by i.v. injection of $^{99m}TcO^-$ (185 MBq) and acquisition of dynamic images for 32 min using a 128 × 128 matrix. Lemon juice (2 ml) was given 16 min after the radiopharmaceutical injection to evaluate salivary excretion. Time activity curves were generated to evaluate the uptake and secretion pattern. Images were acquired with a double headed gamma camera (Philips Forte, The Netherlands) equipped with a low-energy high-resolution collimator.

Image analysis

Evaluation of each ^{99m}Tc-EDDA/HYNIC-TOC scan was performed visually and semi-quantitatively by two experienced nuclear medicine physicians (KAF and MC), who were unaware of the underlying pathology, patient's clinical history, and the results of the other clinical parameters. Corresponding studies were compared for the final analysis and ruled as matching or mismatching.

The SRS was semi quantitatively analyzed in joints using a scale of 0 to 5 using the uptake in the calf muscle as the background signal where 0 corresponds to no uptake, less than background (T/B < 0.8); positivity was defined by a score from 1 to 5 with a joint having a score of 1 if uptake was detectable but lower than back- ground (T/B between 0.8 and 1.0); 2 if

uptake was similar to background (T/B between 1.0 and 1.2); 3 if uptake was slightly higher than background (T/B between 1.2 and 1.4); 4 if uptake much higher than background (T/B between 1.4 and 1.6); and 5 if uptake was clearly high (T/B > 1.6). This scale provides sufficient stratification to define the severity of the inflammatory process and enables comparison between studies and organs. The scores reported in tables are the average between scores of two readers (KAF and MC), and in particular:

- n = number of positive joints (with a score greater than 1),
- global score = the sum of all joint scores,
- severity index = global score divided by number of positive joints.

The uptake in the salivary glands was also semi quantitatively analyzed as follows:

n = number of positive salivary glands,

global score = sum of all positive glands uptake scores (scores assigned as for joints).In functional salivary gland scintigraphy we evaluated the following parameters:

Uptake score = target to background ratio of sum of activity at 12–16 min in both parotid glands before lemon juice administration (temporal region was taken as background) and at 28–32 min after lemon juice administration.

Functional score = ratio between the uptake score before and after administration of lemon juice.

Statistical analysis

Differences between groups were evaluated by unpaired Student's t test; intra-group variations were studied using paired Student's t test. Regression analysis between different parameters was also performed.

Results

Table 1 shows the demographic characteristics and findings of SRS in joints and salivary glands. All patients showed uptake in joints with a mean global

score of 17.0 (with 8.5 affected joints per patient on average with a range of 1–20); however, only 12 patients out of 18 showed uptake in salivary glands (1 to 4 glands involved with a range of global score from 1 to 6) despite all patients having histologically proven secondary SS.

Table 2 shows the semi quantitative scores of SRS in joints in the 11 patients with pre-and post-therapy evaluation. Radiopharmaceutical uptake was significantly reduced in joints after therapy, with a statistically significant difference in the severity index, global score, and number of positive joints ($p = 0.009$, $p = 0.001$, $p = 0.002$, respectively).

Table 3 shows the semiquantitative scores of SRS and SGS in salivary glands in patients pre and post therapy. SRS did not show any significant reduction of radiopharmaceutical uptake in salivary glands after therapy (mean global score 1.73 +/- 2.1 before therapy vs 1.18 +/- 1.25 after therapy; p = ns), and only three patients showed a mild improvement (patients 1, 8, and 18). SGS after therapy with infliximab was also similar to the scan before therapy (mean functional score 5.96 +/- 0.97 before therapy vs 6.23 +/- 0.91 after therapy; *p* = ns).

ESR and CRP decreased significantly during the treatment period. Infliximab was well tolerated without side effects.

The review of the scans from the database of patients with NETs showed no significant periarticular uptake around knees and shoulders with a grade score of 3 or 2 and a symmetric pattern was observed in 8 out of 20 cases, all of them older than 60 years. Uptake in hands was observed, with a score lower than 3, with a diffuse and asymmetric pattern located solely in the carpal joints. In salivary glands, there was no uptake in 16 out of 20 patients.

Three patients with Hashimoto disease had positive findings in the submaxillary glands but not in the parotids. One patient had positive findings only in parotid with an asymmetric pattern.

Table 1

Demographic characteristics and pre therapy findings on SRS in joints and salivary glands of patients studied.

Patient	Age	Gender	n	Global score	n	Global score
1	18	F	13	23	4	6
2	25	F	4	18	1	2
3	40	F	5	5	2	2
4	32	F	2	2	2	2
5	45	F	1	3	1	2
6	60	F	7	13	0	0
7	22	M	10	15	0	0
8	36	F	4	8	2	4
9	29	F	4	14	0	0
10	42	F	8	14	2	6
11	40	F	10	14	0	0
12	52	F	10	20	0	0
13	54	M	10	24	1	3
14	60	F	12	22	0	0
15	62	F	8	16	2	2
16	64	F	17	29	1	2
17	39	F	20	52	2	1
18	70	F	8	14	2	4
Mean			8.5	17	1.2	1.9
±SD			4.9	11.3	1.1	1.9

Table 1 *Demographic characteristics and pre-therapy findings on SRS in joints and salivary glands of patients studied*

Table 2

Pretherapy and post therapy on SRS in joints

	Pre-therapy scan			Post-therapy scan		
Patient	n	Global score	Severity index	n	Global score	Severity index
1	13	23	1.8	6	8	1.3
3	5	5	1.0	4	4	1.0
4	2	2	1.0	2	2	1.0
5	1	3	3.0	1	1	1.0
6	7	13	1.9	3	4	1.3
7	10	15	1.5	6	7	1.2
8	4	8	2.0	3	4	1.3
9	4	14	3.5	2	5	2.5
12	10	20	2.0	7	13	1.9
14	12	22	1.8	5	6	1.2
18	8	14	1.8	2	3	1.5
Mean	6.9	12.6	1.9	3.7*	5.2**	1.4***
±SD	4.0	7.3	0.7	2.0	3.3	0.4

Table 2 *Pre-therapy and post-therapy on SRS in joints. The table shows values of the number of positive joints (n), of the global score and of the severity index in the joints of the 11 patients that performed somatostatin receptor scintigraphy (SRS) before and after treatment with Infliximab *p = 0.002 vs pre-therapy n; **p = 0.001 vs pretherapy global score; ***p = 0.009 vs pre-therapy severity index therapy severity index*

Table 3

Pre therapy and post therapy findings on SRS and SGS in salivary glands.

	Pre-therapy scan			Post-therapy scan		
	SRS	SGS		SRS	SGS	
Patient	n	Global score	Functional score	n	Global score	Functional score
1	4	6	7.5	3	3	7.5
3	2	2	5	5	2	2.5
4	2	2	4.5	2	2	4.4
5	1	1	5.4	1	1	6.3
6	0	0	6.2	0	0	6.3
7	0	0	5.2	0	0	6.1
8	2	4	5.7	2	2	6.5
9	0	0	6.5	0	0	6.7
12	0	0	7.6	0	0	7.4
14	0	0	6.2	0	0	6.4
18	2	4	5.8	2	3	5.9
Mean	1.18	1.73	5.96	1.09*	1.18*	6.23*
±SD	1.33	2.1	0.97	1.14	1.25	0–91

Pre-therapy and post-therapy findings on SRS and SGS in salivary glands
Table shows values of the number of positive joints (n), of the global score and of the functional score in the salivary glands of the 11 patients that performed somatostatin receptor scintigraphy (SRS) before and after treatment with infliximab
**p = n.s. vs pre-therapy values*

Discussion

Somatostatin receptors, amongst other markers, are known to be expressed in human T lymphocytes that infiltrate tissues in autoimmune diseases. In this study, we have used SRS using a radiolabelled somatostatin analogue (^{99m}Tc-EDDA/HYNIC-TOC) to detect immune mediated processes in patients with rheumatoid arthritis and secondary Sjögren's syndrome. We also evaluated whether positivity to SRS was predictive of therapeutic response and if SRS could be used to monitor immunomodulatory therapy with infliximab. All our patients with arthritis and secondary SS ^{99m}Tc- EDDA/HYNIC-TOC scans showed intense uptake with a symmetric and focal pattern in hands, predominantly in carpal, metacarpal, and proximal interphalangeal joints. This finding agrees with the 2010 ACR/EULAR criteria for diagnosis of RA in the hand (15) (Fig. 1). Other compromised joints were knees, shoulders, and ankles to a lesser degree. The quantitative analysis in all patients showed a mean global score of 17, and in the 11 treated patients the mean fell from 12.6 to 5.2 after treatment with infliximab ($p = 0.001$). Also, the mean se- verity index fell from 1.9 to 1.4 after therapy ($p = 0.009$) (Fig. 2). We found no correlation between joint pain or swelling and SRS positivity. Indeed, some joints that were apparently poorly affected showed high somatostatin uptake and, vice versa, in some painful and swollen joints, we found only a moderate uptake. This finding is in agreement with the theory that somatostatin receptors can be overexpressed in active phases of the dis- ease characterized by endothelial activation and lymphocyte infiltration in the synovial cells (16). Nevertheless, all patients and all positive joints showed a clinical and scintigraphic improvement after infliximab therapy. Thus, it can be assumed that SRS is able to identify patients with active disease responding to anti-TNF-α therapy.

A possible limitation of the present study is that patients with a negative SRS had not been treated with infliximab. Therefore, we cannot conclude that negativity at SRS is related to inefficacy of the therapeutic response. However, we can compare our results with other published studies in which patients were not selected on the basis of SRS positivity and the response to infliximab therapy

had a much lower rate of success (5)This finding supports the hypothesis that SRS could identify patients with active disease who may benefit from infliximab therapy.

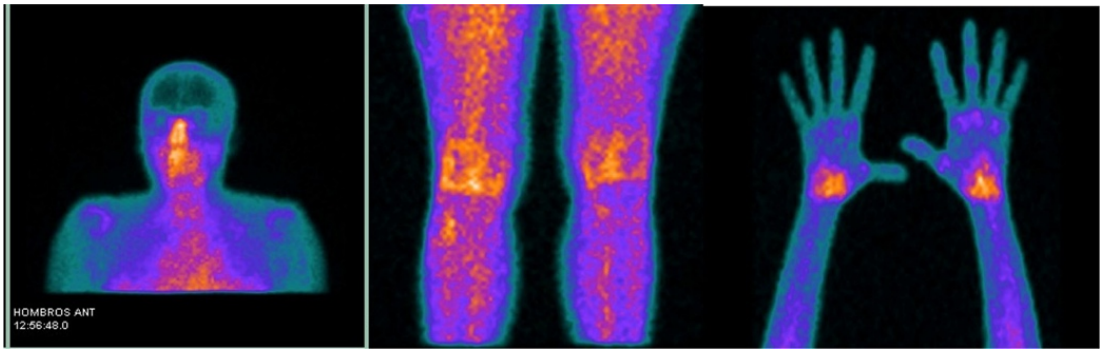

Fig .1 SRS of patient 17, showing high uptake of ^{99m}Tc-EDDA/HYNIC-TOC in sub mandibular glands, shoulders, knees, and hands with symmetrical pattern in carpal and metacarpal joints.

In patients affected by RA, SSTRs are expressed by synovial endothelial cells and synovial macrophages. The SSTR1 and SSTR2 subtypes have been identified on rheumatoid synovial fibroblasts. The inhibitory effects of somatostatin have been documented in cultures of normal activated lymphocytes and of RA synovial cells. Somatostatin not only inhibits proliferation but also suppresses several mediators of inflammation including pro inflammatory cytokines such as TNF-α, IL-1B, and IL-8 in vitro and in vivo (17).Somatostatin and its receptors are produced in macrophages and lymphocytes probably via signaling of growth factors and cytokines, and co-activation has been demonstrated in T cells, granulomatous lesions, and synovial fibroblasts from RA patients (18). In a study of 14 consecutive patients with RA, SRS demonstrated uptake of ^{111}In pentetreotide in inflamed joints with a lesion related sensitivity of 74 % (19).

Adams et al. confirmed the expression of SSTR1 and SSTR3 on inactivated endothelial cells, whereas SSTR2 is strongly expressed after activation (20). Diffuse infiltration of $CD4^+$ lymphocytes and macrophages can always be detected in affected joints, where they express SSTR upon persistent

immunological activity (21). Studies in vitro have shown that proliferation of synovial cells of patients suffering from RA could be inhibited by somatostatin. This may provide a rationale for the therapeutic use of long acting somatostatin analogues for treatment of this disease. In a clinical trial by Paran et al., a significant clinical improvement was observed in patients with refractory RA treated with a long acting somatostatin analogue (22). SRS, therefore, holds important information not only by demonstrating the presence of inflammation but, in positive patients, could also provide a rationale for the treatment of the disease with unlabeled somatostatin.

Our study demonstrated that SRS scintigraphy was positive in all patients and in several different joints but not in all salivary glands. It is important to remark that when the salivary gland pattern of uptake was compared in SRS and SGS, a discrepancy was found in all positive patients. This finding confirmed that the appearance of the salivary glands in SRS is secondary to the presence of somatostatin receptors and not to free $^{99m}TcO^-$, which was reinforced by the fact that the stomach was not visualized in any of the patients (Fig. 3).

The analysis of the scans of patients affected by NETs and without inflammatory disease showed normal mild to moderate uptake of the radiopharmaceutical in the liver, spleen, renal shapes, and gastrointestinal tract. Faint uptake of the radiopharmaceutical was observed in the thyroid gland as reported by Duet et al. [9]. Uptake in the stomach was not observed in any patient, thus excluding the presence of circulating free TcO^-. In some patients (>60 years old) radiopharmaceutical uptake was observed in carpal joints, knees, and shoulders with a symmetrical appearance and a score equal to or lower than 3. Vanhagen et al. postulated that this finding could be explained by osteo degenerative disorders (19). The absence of salivary gland uptake (16 out of 20, 80 %) was a common finding. However, an increased uptake was observed in the salivary glands of three patients and was related to Hashimoto's disease. This might be explained by the fact that the most common thyroid disorder found in association with SS is Hashimoto's thyroiditis and that some antigens are shared by salivary

glands and thyroid gland, which could be responsible for the association between these two pathologies (23).

Pre-Therapy SRS **Post-Therapy SRS**

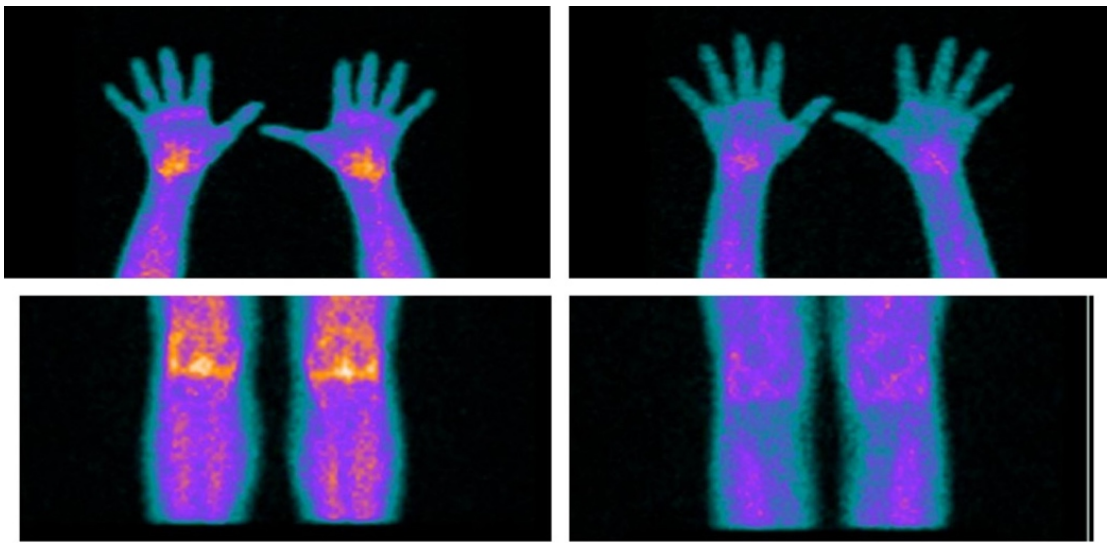

Fig. 2 SRS in patient number 14 before (left images) and after therapy (right images) with infliximab. Global score in hands and knee before therapy was 22. Global score post-therapy was 6, showing good response to treatment.

In our study, only 12 patients of the 18 reported in this study had at least one positive salivary gland at SRS. After therapy with Infliximab (given in 11 patients, see Table 3) only 3 patients showed an improvement of salivary SRS after therapy (1, 8, and 18) and another 3 patients showed a mild improvement at SGS (patients 5, 7, and 8). There was no correlation seen between the findings of the two studies in salivary glands indicating that SRS identifies the inflamed sites mediated by somatostatin that did not necessarily correlate with the structural changes evidenced at SGS. From the clinical point of view, all patients had secondary histologically proven SS, and all patients showed clinical improvement of xerophthalmia and xerostomia after therapy with infliximab. Therefore, SRS may help to identify secondary SS in approximately 67 % of patients with RA but does not help in selecting patients who would respond to anti-TNFα. This finding

agrees with a recent study in patients with primary SS in which the authors showed that anti-TNFα therapy does not have an effect on glandular and extra-glandular manifestations of SS (24). As far as the type of treatment is concerned, to date, there is no standard treatment available for SS and no studies have been performed to evaluate the effect of any therapy on the function of infiltrated salivary glands.

Imaging techniques in SS include many different methods such as ultrasound, MRI, sialography, and salivary gland scintigraphy, but none of these techniques have a high enough sensitivity and specificity to be considered a 'gold standard'. To date lip biopsy of the salivary glands is considered the most reliable diagnostic test and is still the most accurate for diagnosis of SS. Sensitivity and specificity of lip biopsy range from 82 to 95 % and from 75 to 90 %, respectively (25–27). Therefore, only a combination of clinical, immunological, histological, functional, and morphological parameters can help to establish a correct diagnosis of SS and to assess the activity of the disease in order to define the most ap- propriate treatment and to follow-up its efficacy.

In our study, we used a ^{99m}Tc-labelled somatostatin analogue (^{99m}Tc-EDDA/HYNIC-TOC) to evaluate the activity status of joint inflammation and associated salivary gland inflammation in a selected group of patients with RA and secondary SS. The hypothesis behind the study was that SRS could be a useful tool for the workup of these patients for therapeutic decision-making and follow-up of biological therapies. Several hypotheses can be proposed to explain the different behavior of joint and salivary gland disease. Firstly, the two may have a different pathogenesis and natural history. Secondly, our patients were primarily RA patients with long standing active disease and refractory to conventional therapy. The onset of SS might have occurred at different time points. Finally, it is known that secondary SS is very difficult to treat and improvement might be very poor and transient.

A possible limitation of our study could be the small number of cases that were followed up after therapy and by the absence of infliximab treatment of patients

who were negative for SRS. Therefore, we cannot fully assess a correlation between positivity at SRS and therapy response. Notably, however, in other studies in which infliximab therapy had a much lower rate of success, SRS were not used to select patients for therapy (18).

Conclusions

Our pilot study indicates that SRS using ^{99m}Tc-EDDA/ HYNIC-TOC is positive in joints and, to a lesser extent, in salivary glands of patients with RA and secondary SS who do not respond to conventional treatment. SRS may therefore be a useful imaging tool to assess disease activity in RA and help to detect secondary SS. Given that all positive patients showed a benefit from infliximab therapy in the joints, SRS positivity might be considered as a positive prognostic factor. We therefore suggest the use of SRS for the selection of refractory RA patients who may be treated with biological therapies but not as a tool for defining treatment in secondary SS.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

LKAF and MC performed all scintigraphic studies and read the images. FG and AWJMG participated in the design of the study and performed statistical analysis. SMM, VT and AM deal with patient management. AS conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

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Chapter 5

Value of Somatostatin receptor scintigraphy with ^{99m}Tc-HYNIC-TOC in patients with primary Sjögren's Syndrome.

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Abstract:

Objectives. Primary Sjögren's syndrome (SS) is diagnosed based on the American European Consensus Group (AECG) criteria, but lacks specificity, not only in the involvement of salivary glands, but also in extra-glandular involvement. Whole body somatostatin receptor scintigraphy with ^{99m}Tc-HYNIC-TOC scintigraphy could overcome these limitations. The aims of this study were to evaluate salivary gland

uptake of ^{99m}Tc-HYNIC-TOC in untreated patients with de-novo diagnosis of SS as compared to control subjects and as compared to conventional sialoscintigraphy with $^{99m}TcO_4^-$. We also aimed to evaluate the involvement of joints. *Methods.* ^{99m}Tc-HYNIC-TOC was performed in SS patients and uptake in joints and salivary glands was analyzed semi-quantitatively. Patients also underwent a $^{99m}TcO_4$ sialoscintigraphy. The control group that we analyzed consisted of 30 patients with neuroendocrine tumors. *Results.* Fifty-two females and ten males fully met the AECG criteria for SS, and were included. Target Background Ratio (TBR) >1.18 in submandibular glands correctly classified 93% of the patients with SS in comparison to 27% for $^{99m}TcO_4$ sialoscintigraphy. The area under the curve of the ROC analysis for TBR in submandibular glands was 0.95. In joints there was a huge variety in uptake. The median TBR was significantly higher in salivary glands in patients with SS compared to controls. *Conclusion*. ^{99m}Tc-HYNIC-TOC scintigraphy identified active inflammatory processes not only in the salivary glands, but, unexpectedly, also in many joints in patients with primary SS, contrary to popular believe. This technique provides an objective parameter to evaluate the inflammation burden in salivary glands and joints and could be used to evaluate response to treatment.

Keywords: Sjögren's syndrome; ^{99m}Tc-HYNIC-TOC; somatostatin receptor scintigraphy; inflammation; salivary glands

Introduction

Sjögren's syndrome (SS) is a systemic autoimmune disease that primarily affects the salivary and lacrimal glands. It usually causes a persistent dryness of the mouth and eyes due to lymphocytic infiltration and impairment of the exocrine glands (1,2).The presence of circulating auto antibodies that evoke an autoimmune response by cytokines derived from both T and B cell lymphocytes are thought to contribute to the inflammation and destruction of the glandular tissue (3). Primary SS is characterized only by the presence of these exocrinopathies whereas in secondary Sjögren these disorders are associated with other autoimmune diseases (1).

Primary SS has a prevalence of about 0.5% in the general population, with a female predominance of 9:1, which is approximately similar to Systemic lupus erythematosus (SLE) (4). SS is commonly included in the spectrum of connective tissue diseases and sometimes shows multisystemic involvement with a large range of clinical and serological manifestations. Besides the disease-specific exocrine manifestations, SS may be characterized by the involvement of the joints, skin, lung, kidneys and nervous system and it is associated with the production of a variety of autoantibodies (5). The American–European Consensus Group (AECG) criteria, published in 2002 (6) was adopted as the gold standard criteria in Europe and in the United States to diagnose SS. For primary SS, the presence of four out of six items showed good sensitivity (93.5%) and specificity (94%) (7,8). Nowadays, the ACR-EULAR initiative decided to reunite the criteria to make clinical studies and therapeutic trials comparable(9). In this new approach, the sialoscintigraphy is not included as diagnostic criterion. As a matter of fact, although sialoscintigraphy was considered part of the diagnostic criteria for SS for AECG, this is a technique that lacks specificity and is not commonly used anymore (10). Diagnosing secondary SS has not yet been addressed by the AECG, however, in practice it is usually required to fulfill the criteria for primary SS and to additionally fulfill the American College of Rheumatology (ACR) criteria for an established connective tissue disease such as Rheumatoid arthritis (RA), SLE, dermatomyositis, myositis, or biliary cirrhosis(11). Especially in the clinical diagnostic setting where SS patients present with severe

dryness, positive autoantibodies, and positive lip biopsies, it is important to assess the extent of extra glandular involvement for therapy decision making. Therefore, a standardized whole body imaging technique to determine other sites of disease manifestations is highly needed. Somatostatin is a hormone that regulates several physiological cell processes via specific receptors expressed throughout the body, particularly by nerve cells, many neuroendocrine cells and cells mediating inflammation and the immune response (12). Its physiological actions are initiated by binding to G-protein-coupled somatostatin receptors (SSTR1-SSTR5) (13).High expression levels of SSTRs have been observed in tumor cells as well as neo-angiogenic and peritumoral vessels, epithelioid cells, proliferating synovial vessels and activated lymphocytes and monocytes (14). Besides overexpression in several autoimmune and granulomatous diseases, such as RA, SLE, Schönlein-Henoch, autoimmune uveitis, ulcerative colitis, sarcoidosis, tuberculosis and Crohn's disease, SSTR overexpression is also well known in patients with SS (15,16).

A strong interest in SSTRs as targets for in vivo diagnostic and therapeutic purposes followed the availability of somatostatin analogues (17).Several molecules that bind to SSTR2 and SSTR5 receptors isoforms, and with lower affinity to SSTR3 (18) have been labelled with ^{111}Indium (^{111}In-octreotide[19] and ^{111}In-DTPA-D-Phe(1)-octreotide (OctreoScan®, Mallinckrodt). In order to overcome some limitations in the use of OctreoScan®, including the high costs and suboptimal physical features of ^{111}In, somatostatin analogues have also been labelled with ^{99m}Technetium such as Depreotide (15) and ^{99m}Tc–EDDA/Tricine-HYNIC-Tyr(3)-Octreotide (^{99m}Tc-HYNIC-TOC) (19).The latter has recently been used in clinical settings including neuroendocrine tumors (NET) and a number of chronic inflammatory diseases (20-22) where uptake of the tracer was described, not only in the main compromised organs, but also in the salivary glands. ^{99m}Tc-HYNIC-TOC has a high affinity for SSTR2, 3 and 5 and has demonstrated potential utility in the diagnostic work-up and treatment evaluation of chronic inflammatory diseases (22). Although the use of ^{99m}Tc-HYNIC-TOC has been extensively described for malignancies (15) and chronic inflammatory processes, including secondary SS (7,17), the diagnostic,

prognostic and therapeutic potential in primary SS has, to our knowledge, not been previously addressed.

The main objective of this study was to evaluate the characteristics of ^{99m}TcHYNIC-TOC distribution in the salivary glands of patients with newly diagnosed SS based on a semi-quantitative analysis. The secondary objectives were (a) to correlate our findings in the salivary glands to the conventional sialoscintigraphy with $^{99m}TcO_4^-$, (b) to evaluate extra-glandular involvement of the joints, and (c) to compare the findings in salivary glands and joints with control patients without SS that underwent ^{99m}Tc-HYNIC-TOC scintigraphy.

Materials and Methods

Study design

We retrospectively analyzed a consecutive cohort of 62 patients with de novo diagnosis of primary SS who underwent ^{99m}Tc-HYNIC-TOC scintigraphy at the nuclear medicine unit of Clinica Colsanitas in Bogotá, between January 2013 and November 2016. Furthermore, regarding the negative control group, we evaluated the uptake of salivary glands uptake and joints uptake of ^{99m}Tc-HYNIC-TOC in 30 patients in whom the scan was performed for staging of NET. In order to avoid an influence on salivary gland uptake in this control group population, only subjects with negative scans or with a very low tumor burden (located only in the abdominal area) were chosen.

Radiopharmaceutical

^{99m}Tc-HYNIC-TOC was prepared in the radiopharmaceutical department from a commercially available kit (Tektrotyd®, POLATOM, Otwock, Poland) in accordance with the manufacturer's instructions. Briefly, freshly eluted $^{99m}TcO_4^-$ (740 MBq) in a 0.9% NaCl solution (pH 7) was added to the vial containing HYNIC-Tyr3-Octreotide (20 µg), tricine and EDDA, mixed and incubated at 80°C for 30 minutes according to existing recommendations (23).

Imaging procedures

^{99m}Tc-HYNIC-TOC scintigraphy

Static planar spot view images of the whole body were performed to evaluate the involvement of salivary glands and major and minor joints. Each spot view image was acquired for 10 minutes starting 3 hours after intravenous (i.v.) injection of ^{99m}Tc-HYNIC-TOC (approximately 370 MBq) using a 512x512 matrix. The day before the study, the patients were given oral Lugol solution to prevent the uptake in salivary glands of free $^{99m}TcO_4^-$ possibly released by the catabolism of ^{99m}Tc-HYNIC-TOC. The acquisition protocol used was exactly the same as described earlier (22) .

$^{99m}TcO_4^-$ Sialoscintigraphy

Functional salivary gland scintigraphy (sialoscintigraphy) was performed by i.v. injection of $^{99m}TcO_4^-$ (185 MBq). Images were acquired dynamically over the course of 30 min using a 128x128 matrix. Lemon juice (2 ml) was given 10 minutes afterwards in order to stimulate salivary excretion. Time activity curves were generated to evaluate the uptake and secretion patterns.
All scintigraphic images were acquired with a double-headed gamma camera (Infinia, General Eletrics, USA) equipped with a low energy, high-resolution collimator in accordance with a previously described protocol (22).

Image analysis

The ^{99m}Tc-HYNIC-TOC images were analyzed by two observers (KA and JR) independently of each other and blinded to clinical details. Analysis was performed by using a method proposed before (21). Briefly, for semi-quantitative analysis, the calf uptake was used as the reference background. A small region of interest (ROI) was delineated and duplicated for each salivary gland and joint bilaterally: shoulders, elbows, wrists, metacarpophalangeal joints, inter-phalangeal joints, knees, and ankles. We did not consider the hips because of possible artifacts due to high bladder activity. By using the average counts in the ROIs, we calculated a ratio to

compare and analyze the findings, according to this formula: Average counts in the ROI in each part of interest/Average counts in the calf, leading to a target-to-background ratio (TBR).

Regarding the functional salivary gland scintigraphy, the following parameters were evaluated: (i) uptake score = TBR of sum of activity at 6-10 minutes in both parotid glands before lemon juice administration (temporal region was taken as background) and at 24-28 minutes, after lemon juice administration; and (ii) functional score = ratio between the uptake score before and after administration of lemon juice according to Schall et al.(24).

Statistical analysis

Full descriptive analysis of the variables of interest was performed in order to comply with the objectives of the study. Frequencies (absolute and relative) and percentages were calculated for the qualitative variables and measures of the central tendency (mean and median) and dispersion (standard deviation and interquartile range) together with the maximum and minimum values for the quantitative variables. A stratified analysis of the TBRs in the control group and also in the patients with SS was performed. Mann-Whitney test was used to evaluate the differences in the TBRs values in the salivary glands between control patients and SS population. A posthoc analysis revealed a power of 0.85 with the sample that was used. The analysis was performed with the Stata 14.2 SE program.

Results

Patient groups

Data was gathered from 62 patients with confirmed primary SS and 30 healthy control subjects. Demographic characteristics of the population and the frequency of symptoms are summarized in table 1. It is remarkable how much the frequency of the symptoms and the positive laboratory tests results that belong to AECG criteria vary. The salivary gland histopathology, the anti-SSa and anti-Ro were present in 77%,62% and 62%, respectively. Moreover, the high frequency of joint pain (87%) as part of the symptoms which are not included as diagnostic criteria in AECG, is remarkable. The ^{99m}Tc-sialoscintigraphy was positive in 27% of patients.

Table 1. Demographic characteristics for the total population.

	n	**%**	**n**	**%**
Gender Female	22	73.3	52	83,87
Age;Median (range)	58.5	64 (16-80)	48.5	48 (15-71)
12-18 years	1	3,33	1	1,61
19-40 years	1	3,33	15	24,19
41-60 years	9	30,00	34	54,84
>60 years	19	63,34	12	19,35
Dry eye*	-	-	60	96,77
Dry mouth*	-	-	60	96,77
Schirmer test*	-	-	49	79,03
Msg histopathology*	-	-	47	75,80
Sialoscintigraphy*	-	-	17	27,42
Joint pain	-	-	54	87,10

AECG criteria*. Abbreviations: Msg histopathology: minor salivary gland histopathology.

Salivary glands uptake of ^{99m}Tc-HYNIC-TOC

The analysis of the TBR obtained for the salivary glands for each group of patients showed higher values in submandibular glands in patients with primary SS with a median of 2.73 and a maximum of 5.11. The median for control patients was 1.09. Regarding the parotid glands, the median and maximum recorded values of TBR were 1.72 and 2.3, respectively. A significant higher TBR of the salivary glands was found in patients with primary SS compared to the control group ($p<0.001$, Mann-Whitney test). The sensitivity/specificity ROC curve for the TBR in submandibular glands was 0.95(CI: 0.91-0.98);we found that a TBR>1.18 in submandibular glands correctly identified 92% of the patients with primary SS.

Figure 1 highlights the differences regarding the median values of TBRs for salivary glands between the control group and the primary SS group.

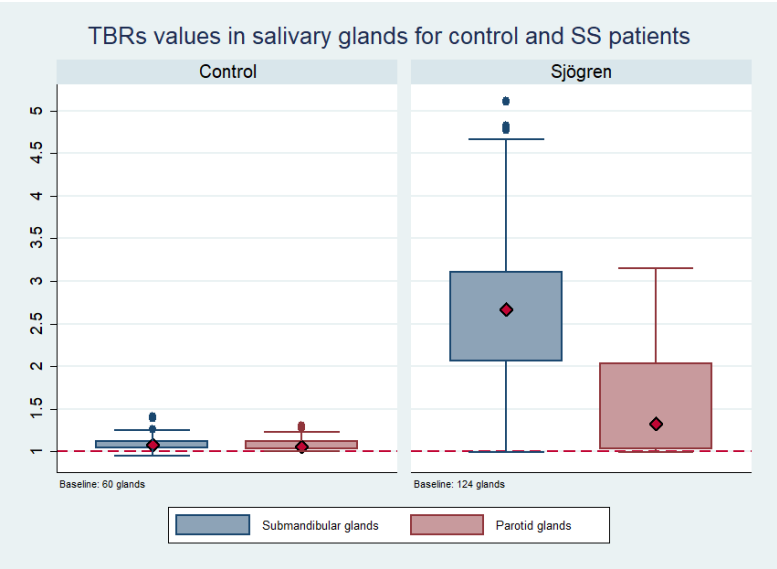

Figure 1. TBR in salivary glands of primary SS patients and control subjects.

<u>Comparison between ^{99m}Tc-HYNIC-TOC in salivary glands and ^{99m}Tc-sialoscintigraphy.</u>

When we analyzed the uptake of $^{99m}TcO_4^-$ in sialoscintigraphy we found that only 17 patients with primary SS (27%) showed abnormal findings vs 60 patients (97%) who visually showed any grade of uptake with ^{99m}TcHYNIC-TOC. With semi-quantitative analysis, using a TBR > 1.18 as cut-off point, we found that 57 patients (92%) were identified correctly by ^{99m}Tc-HYNIC-TOC.

Joint uptake of ^{99m}Tc-HYNIC-TOC

Table 2 shows a descriptive analysis of the TBRs for every single joint in both groups.

Table 2. TBRs description for joints in SS and control patients.

TBR values for joints in control patients						TBR values for joints in SS patients				
	n	median	SD	min	max	n	median	SD	min	max
Carpus	60	1.02	0.16	0.30	1.70	124	2.92	1.09	0.33	5.50
Metcp	60	1.10	0.13	0.13	1.70	124	1.06	0.57	0.90	3.20
Intphpr	60	1.00	0.13	0.98	1.70	124	1.02	0.14	0.90	1.60
Intphd	60	1.00	0.13	0.96	1.70	124	1.02	0.10	0.90	1.20
Shoul	60	1.25	0.25	0.95	1.90	124	1.03	0.40	0.90	2.10
Knee	60	1.10	0.15	0.98	1.70	124	2.60	1.30	0.90	5.60
Ankle	60	1.02	015	0.97	1.70	124	1.00	1.00	0.90	5.30
Elbow	60	1.02	0.15	0.94	1.70	124	1.10	0.70	0.90	4.10

Abbreviations: Metcp: metacarpophalangeal. Intphpr: proximal interphalangeal joint. Intphd: distal interphalangeal joint. Shoul: shoulder.

In patients with SS the highest values were found in the carpus, followed by the knees while the lowest values were recorded in the distal interphalangeal joints (Figure 2).

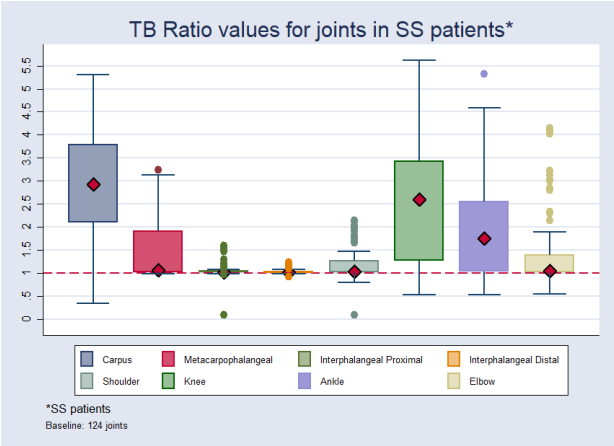

Figure 2. TBRs values for joints in patients with SS.

Figure 3 shows an example of two patients with SS disease with different degrees of uptake of 99mTc-HYNIC in salivary glands (arrows), carpus and knees.

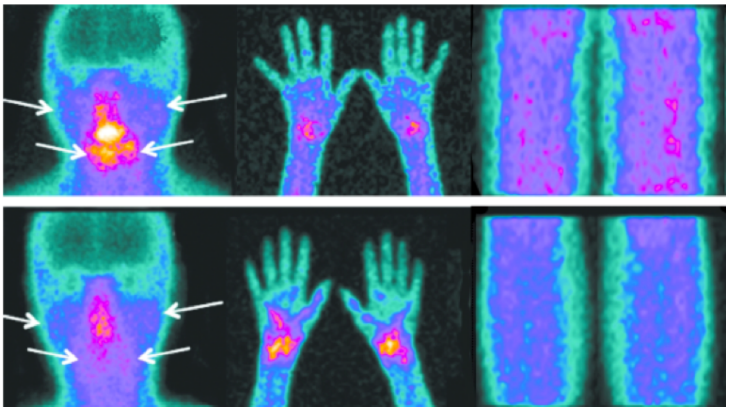

Figure 3. Example of two scans in two patients showing different degrees of ^{99m}Tc-SST uptake in the parotid and submandibular glands (arrows), carpus (both patients positive) and knees (both patients negative).

Discussion

This study evaluated, for the first time, the distribution of ^{99m}Tc-HYNIC-TOC in salivary glands and joints in a population of patients with untreated primary SS. Since SS is an immunological disorder that may involve also involve other organs and tissues besides the salivary glands, this whole body imaging technique was able to identify the involvement of the joints. For salivary glands, this imaging modality was found to be better than sialoscintigraphy, which although is part of the AECG criteria to diagnose PSS, nowadays it tends not to be considered as part of the diagnostic criteria. ^{99m}Tc-HYNIC-TOC could potentially replace sialoscintigraphy in the diagnostic and prognostic criteria for evaluation of this disease.

In our population the most prevalent symptoms were keratoconjunctivitis, xerostomia and joint pain, which are findings frequently reported in the literature as part of a broad variety of clinical manifestations and biological abnormalities. Moreover, it is well known that this variety in symptoms accounts for the delay in diagnosis (25). The frequencies of symptoms and signs observed in our population with respect to the AECG criteria confirmed the importance of combining them for early diagnosis.

Likewise, the observed incidence of positive studies in conventional sialoscintigraphy in our population (27%) was low in comparison to the literature. This may be caused by the fact that the distinction between normal results and minor dysfunction is not always easy to detect and mild glandular impairment and borderline results may be misclassified by subjective judgment. In addition, it is possible that when diagnosis is performed during the early stages of the disease, a large functional compromise of the gland may not be present at all yet. Therefore, the pathophysiological process, which is mediating the disease cannot be accurately evaluated with conventional sialoscintigraphy.

To evaluate the pattern of ^{99m}Tc-HYNIC-TOC uptake, a semi-quantitative analysis was performed with the help of TBR, using the calf as background area. Values were higher in the submandibular glands than in the parotid glands thus showing a more severe involvement of the submandibular glands in our population. Surprisingly, when we compared the TBR of the submandibular glands between primary SS patients and healthy controls, medians were close to 1 for the healthy control patients and above 2.5 for the SS patients with statistically significant difference ($p<0.01$). The ROC analysis showed that a threshold of 1.18 allowed to correctly identify 92% of the patients. Only two patients showed no uptake of ^{99m}Tc-HYNIC-TOC, these patients had serological and ocular test positive for SS with sicca symptoms; presumably, the disease was not affecting the salivary glands yet and therefore activated their immune mechanisms. Given the mechanism of action of this radiopharmaceutical our results show its ability to detect the presence of somatostatin-mediated immune cell activation in salivary glands in patients with primary SS. The theoretical model reported in the literature supports the usefulness of this technique: abnormal antibodies and T cell responses to muscarinic type 3 receptors (M3R) have been conceived to be pathogenic in primary SS patients (26) The presence of autoantibodies against M3R has been reported, and it suggests that an immune reaction to M3R reactive T cells have been detected in the generation of SS (27). M3R reactive T cells have been detected in 40% of patients with SS, suggesting that the M3R immune response in SS might function as an autoantigen recognized by autoreactive T cells (28). Patients with SS have long been

thought to suffer from a M3R reactive T cells type Th1 condition, which has been supported by high levels of IFN-gamma in the serum and a predominance of Th1 over Th2 cells in the blood. Despite a number of caveats, SS is currently conceived as a model for B cell-induced autoimmune disease (29). It is known how the activities of these cells are orchestrated by soluble factors of the TNF family, most notably the B cell-activating factor (BAFF) described in the late 1990s (30). This immunologic setting could explain the ability of ^{99m}Tc-HYNIC-TOC to detect the inflammatory process, but also the lack of sensitivity of sialoscintigraphy to detect the disease in SS. Although further experimental evidence is needed to confirm this hypotheses, our results suggest that positivity to ^{99m}Tc-HYNIC-TOC may forego a reduction of salivary function. Analyses of gene expression profiles of salivary gland tissue from SS patients have confirmed the presence of chronic inflammation (31) and in vitro analysis suggests that cytokines such as interleukin-1-alpha may affect the process of saliva secretion by inhibiting the release of acetylcholine from cholinergic nerves (32). In this pathophysiological setting of the syndrome, where an immune-mediated inflammatory process exists, the lack of correlation between the two radiopharmaceuticals is not surprising. While ^{99m}Tc-HYNIC-TOC binds to activated lymphocytes and provides information about disease activity, $^{99m}TcO_4^-$ evaluates the functional impairment of glandular parenchyma. Furthermore, although noninvasive imaging techniques such as ultrasonography (US), CT and MR (33) are being studied and might prove useful in the evaluation of the oral involvement in SS, US demonstrated to have a high diagnostic accuracy in identifying structural changes in salivary glands (34) .We believe that ^{99m}Tc-HYNIC-TOC scintigraphy could have an added value since it is capable to demonstrate active inflammatory processes in salivary glands secondary to SS, and could potentially be used for therapy follow-up as well.

The evaluation of the ratios at the level of the joints in SS patients showed that higher median values were recorded in the carpus and knees. When the medians for the different joints of the SS patients and healthy subjects were compared, a significant difference between the two groups was found, with healthy patients having medians close to 1 and SS patients above 2 in knees and carpus. This finding could also

support the theory that somatostatin receptors can be overexpressed in the active phases of the diseases characterized by endothelial activation and lymphocyte infiltration of the synovial cells (35-36). Moreover, we already demonstrated in a pilot study in a RA population how all patients who showed positive findings in joints at the baseline ^{99m}Tc-HYNIC-TOC scan improved after infliximab therapy (22). We have also suggested how this molecule is able to identify patients with active disease responding to anti TNF-α therapy. We could not establish a cut-off in the ratios to describe abnormality in the joints. ^{99m}TcHYNIC-TOC can identify inflamed joints in patients with SS since this is a disease in which the same immunological process at the level of the salivary glands can also be observed around the epithelial structures of other organs including the liver, kidneys and lungs. Moreover, half of the patients develop extraglandular complications including arthritis, interstitial lung disease, nervous system involvement, or tubular nephropathy (37).

We found as limitation a source of confusion bias because we did not control the confounders for the control group (age, gender among others). The analysis of the salivary glands using the planar technique seems to underestimate their appearance and, therefore, SPECT/CT should be the technique of choice for future studies.

One of the strongest point of our study is that our results were the product of a highly trained rheumatologist and nuclear medicine physicians who used highly controlled and standardized methods to evaluate the patients and to decrease the index test bias. We included a representative sample size and we used a strict evaluation judgement to determine the true positive SS patients.

As we know that PET tracers have several advantages over SPECT tracers (better resolution, possibility for absolute quantification) we believe that ^{68}Ga-labelled somatostatin receptor scintigraphy will eventually replace the radiopharmaceutical we used in this study. Nevertheless, our findings are still important because the behavior of the ^{68}Ga-labelled compound will be the same as the ^{99m}Tc-labelled radiopharmaceutical.

Conclusions

In conclusion, we showed that ^{99m}Tc-HYNIC-TOC is an important imaging technique to study SS patients, since it allows us to identify active inflammatory processes not only in the salivary glands, but also in the joints. More studies are required in order to include this imaging modality as part of the diagnostic workout of patients with suspected SS to better define the disease activity and the extent of extraglandular inflammation. Moreover, it may provide an objective parameter to evaluate the response to treatment.

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Chapter 6

Uptake pattern of ^{68}Ga- DOTA-NOC in tissues: implications for inflammatory diseases

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Abstract :

Objectives: ^{68}Ga-DOTA-NOC binds to somatostatin receptor (SSTR) subtypes 2 and 5, also expressed on lymphocytes and macrophages, but no information is available about uptake in tissues that might be affected by a chronic inflammatory process. Our aim was to obtain normal reference values for :^{68}Ga-DOTA-NOC uptake in tissues prone to chronic inflammation. *Methods:* Retrospective study in 81 patients

in whom the scan was performed for a suspicion of neuroendocrine tumor (NET). We analyzed major joints, salivary glands, thyroid, aortic wall from images acquired after injection of 173,9±1 Mbq of :^{68}Ga-DOTA-NOC. We calculated the SUV_{max} and $SUV_{target}/SUV_{gluteus}$ ratio or SUV_{target}/SUV_{aorta} ratio. Data are reported as mean±2 or ±3 standard deviations (SD).

Results: SUV_{max} appeared more reliable than other ratios. In thyroid we found a mean SUV_{max} of 1.36±0.45, with no values >3SD; in parotid glands 0.98±0.40, with 2 values >3SD; in submandibular glands 0.99±0.37, with 2 values >3SD; in aortic arch 1.71±0.50, with 1 value >3SD; in thoracic aorta 2.03±0.52, with 1 value >3SD; in abdominal aorta 2.19±0.49, with no value >3SD; in shoulders 0.92±0.31 and in hips 0.87±0.34, with 2 and 4 values >3SD, respectively. These 12 values with SUV_{max} >3SD, belong to 5 patients, 3 of which had signs of xerostomia and/or arthritis. A statistically significant correlation was observed between SUV_{max} and age in all examined tissues but in the aorta. *Conclusions:* Tissues in which lymphocytic infiltration may occur show that SUV_{max} is tissue-dependent. Within tissue variability, an SUV_{max} higher than the mean +3SD is rarely found amongst patients without a symptomatic chronic inflammatory process but, when found, may highlight a chronic inflammatory condition.

Keywords: somatostatin receptors, ^{68}Ga-DOTA-NOC, inflammation, autoimmune diseases

Introduction

Somatostatin (SST) is a neuropeptide with a very short lifespan secreted in the central nervous system, endocrine glands and gastrointestinal tract where it acts primarily to inhibit neuro-transmission. It interacts with five different subtypes of somatostatin receptors (SSTR 1-5) that belong to the G-coupled seven transmembrane spanning receptor domain family and they are expressed on the surface of many cells and organs (1).

Nuclear medicine offers a panel of radiopharmaceuticals for both gamma-camera and PET studies that provide functional information on body distribution of SST. Small structural modifications, chelator substitution or metal replacement, are known to affect the binding affinity of the molecule to the receptors (2). These radiopharmaceuticals are currently used for diagnosis and follow-up of different pathological conditions characterized by an overexpression of SSTR on their cell surface. The use of radiolabelled SST analogues for diagnosis and therapy follow-up of NETs is nowadays well consolidated and also there is evidence about the utility of these methods in different clinical settings(3) , in particular chronic inflammatory diseases not only in the diagnostic approach but in monitoring therapy outcome (4). Somatostatin receptor scintigraphy (SRS), mainly using SST analogues labeled with Indium-111 or Tc-99m, have been extensively performed in diagnosis and follow-up of chronic inflammatory diseases such as Rheumatoid Arthritis (RA), Sjögren's Syndrome and others (5) , in addition to NETs (6,7), despite normal values of SST uptake in non-neoplastic tissues is poorly known.

In the last 16 years, the better anatomical detail, spatial resolution and diagnostic accuracy of PET/CT, together with the availability of Ge-68/Ga-68 generators, has lead to the development of ^{68}Ga conjugated peptides (-NOC, -TOC, -TATE) that differ for the affinity to SSTR subtypes: ^{68}Ga-DOTA-TOC is more selective for receptors 2 and 5, ^{68}Ga-DOTA-TATE for type 2 receptor (8-10) and ^{68}Ga-DOTA-NOC shows high affinity for SSTR-2 and 5 (11).

Because it has been observed that SSTR-2 and 5 are over-expressed by inflammatory cells (12), radiolabelled DOTA-NOC results an attractive molecule for imaging inflammatory conditions. Despite normal biodistribution pattern of DOTA-

NOC has been extensively described for tumour conditions little information is available about uptake values in tissues that may be potentially affected by an inflammatory diseases (13). Prasad et al. (14) evaluated the uptake in normal organs and tumour lesions with ^{68}Ga-DOTA-NOC in 89 patients with neuroendocrine tumours. Among the examined tissue they evaluated SUV_{max} in thyroid (3.4 ± 1.4). Boy and colleagues (15) studied 120 patients with ^{68}Ga-DOTA-TOC and they described the mean SUV_{max} value for thyroid and parotid glands and found statistically significant gender difference in thyroid. Castellucci et al. measured the extra tumoral uptake of ^{68}Ga-DOTA-NOC only in the pancreatic head and found large variability amongst patients (16).

As the demand of ^{68}Ga-DOTA-NOC PET studies increase for inflammatory conditions, it becomes important to know the normal distribution pattern of this molecule and to quantify the uptake in tissues that can potentially be affected by a chronic inflammatory condition in order to correctly interpret the images in patients with such disorders.

The aim of this study was, therefore, to evaluate the uptake (SUV_{max}) of ^{68}Ga-DOTA-NOC in normal tissues that could be potentially involved in chronic inflammatory diseases to identify a cut off value for SUV_{max} that could help to differentiate between physiologic or pathologic conditions. In particular we measured the ^{68}Ga-DOTA-NOC uptake in parotid glands, submandibular glands (sites of sialoadenitis), in thyroid (site of thyroiditis), in shoulders and hips (sites of chronic arthritis), aortic wall (site of large vessel vasculitis and atherosclerosis).

Materials and methods

Study design

We conducted a retrospective observational study including 84 studies performed with ^{68}Ga-DOTA-NOC PET/CT in the Nuclear Medicine Unit of S. Andrea Hospital, "Sapienza" University of Rome between May 2012 and April 2013.

Study population

Inclusion criteria for this study were: patients with a recent ^{68}Ga-DOTA-NOC PET/CT scan; patients without specific symptoms or clinically diagnosed dysfunction of salivary glands, thyroid, joints and large vessels; age between 30 and 80 years. Exclusion criteria were: patients with diffused neoplastic disease; patients with metastatic cancer adjacent to one of the examined tissues.

All patients had no anamnestic record of autoimmune/inflammatory conditions affecting the salivary glands, thyroid, joints or large vessels.

Study protocol

The Ga-68 was eluted from a Ge-68/Ga-68 generator and bound to DOTA-NOC (ABX, Austria) in compliance with the GMP regulations. Whole body scans were acquired with a dedicated hybrid PET/CT tomograph (Phillips) one hour after intravenous injection of 173.9±1 Mbq of :[68Ga]Ga-DOTA-NOC. PET images were acquired for 3-4 minutes per bed position from head to mid-thigh. A low-dose CT scans for attenuation correction and anatomic location was also performed. Images were downloaded from the hospital database and interpreted as positive or negative by 3 expert nuclear medicine physicians (LKA, CL, CEG).

In all images we calculated the maximum standardized uptake value (SUV_{max}), (T/B) ratio using gluteus muscle ($SUV_{target}/SUV_{gluteus}$) or the lumen of thoracic aorta (SUV_{target}/SUV_{aorta}) in several tissues that could potentially be involved by a chronic inflammatory/autoimmune process.

In particular, salivary glands (parotid and submandibular) because of potential sialoadenitis; thyroid because of thyroiditis; thoracic and abdominal aorta because of potential large vessel vasculitis; shoulder and hip joints for potential chronic arthritis. In symmetrical organs the SUV_{max} were calculated for both left and right side.

The obtained values from the three methods were compared between them.

Statistical analysis: demographics and descriptive statistics were analyzed using Stata version 14. The association between SUV_{max} or T/B values and the age were explored with a multiple linear regression with categorical variables model.

Differences between gender, treatment, and positivity of the scan, were analyzed by Student t test. Two and three standard deviations above the mean identified values above the 95th and 97.5th percentile respectively. A SUV_{max} or T/B value was considered abnormal if it was greater than the mean +3SD.
End point: to identify a SUV_{max} or $SUV_{target}/SUV_{gluteus}$ or SUV_{target}/SUV_{aorta} cut-off as reference for each tissue to be used to identify pathological tissues in future studies in patients affected by chronic inflammatory processes.

Results

We analyzed 84 patients who performed ^{68}Ga-DOTA-NOC PET/CT between May 2012 and April 2013 because of a confirmed or suspected neuroendocrine tumour. Three patients with diffuse neoplastic disease that could potentially interfere with SUV_{max} calculation in tissues due to a "partial volume effect" were excluded from the study. Out of the recruited 81 patients, 9 had previous thyroidectomy and therefore the uptake in thyroid was not available. Main results are summarized in tables 1 and 2. Average age of patients was 57.5 ± 13.2 years and 56.8% were females. Gastro-entero-pancreatic neuroendocrine tumours were the most frequent indication for the nuclear medicine study (58.1%), followed by lung carcinoids (14.8%). In 42 patients (51.9%) the scan was considered positive for pathologic NETs-associated expression of SSTRs and 39 patients (48.1%) were completely normal. Twenty-two patients were under treatment with long-acting somatostatin analogues.

Table I

Patients' clinical characteristics

Patient's data (n=81)	
Mean age ± standard deviation	57.5 ± 13.2
Sex	
Females	46 (56.8%)
Indication	
Gastrointestinal tumour	28 (34.6%)
Pancreatic tumor	19 (23.5%)
Lung carcinoid	12 (14.8%)
Unknown NET	7 (8.6%)
Others	4 (4.9%)
MEN1	2 (2.5%)
Thyroid medullary cancer	9 (11.1%)
Patients with positive scan	42 (51.9%)
Patients in therapy with SST analogues	22 (27.1%)

Table II

Tissues with [68Ga]Ga-DOTA-NOC uptake greater than mean +2SD or +3SD

	SUV_{max}	SUV_{max} >2SD <3SD	SUV_{max}/ $SUV_{gluteus}$ >2SD <3SD	SUV_{max}/ SUV_{aorta} >2SD <3SD	SUV_{max} >3SD	SUV_{max}/ $SUV_{gluteus}$ >3SD	SUV_{max}/ SUV_{aorta} >3SD
Tissue	mean (SD) median [range]	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Hips (n=81)	0.87 (0.35) 0.82 [0.37-2.42]	4 (4.9%)	4 (4.9%)	2 (2.5%)	4 (4.9%)	2 (2.5%)	3 (3.7%)
Shoulders (n=81)	0.92 (0.32) 0.88 [0.28-2.48]	6 (7.4%)	5 (6.2%)	3 (3.7%)	2 (2.5%)	1 (1.2%)	2 (2.5%)
Parotid glands (n=81)	0.99 (0.40) 0.91 [0.22-2.70]	3 (3.7%)	6 (7.4%)	4 (4.9%)	2 (2.5%)	0 (0%)	1 (1.2%)
Submandibular glands (n=81)	0.99 (0.37) 0.96 [0.02-2.55]	1 (1.2%)	1 (1.2%)	5 (6.2%)	2 (2.5%)	2 (2.5%)	1 (1.2%)
Thyroid (n=72)	1.36 (0.45) 1.34 [0.28-2.66]	5 (6.9%)	4 (5.5%)	4 (5.5%)	0 (0%)	2 (2.7%)	1 (1.4%)
Aortic arch (n=81)	1.71 (0.50) 1.71 [0.53-3.46]	1 (1.2%)	1 (1.2%)	2 (2.5%)	1 (1.2%)	0 (0%)	1 (1.2%)
Thoracic aorta (n=81)	2.03 (0.52) 2.01 [0.99-3.89]	2 (2.5%)	1 (1.2%)	0 (0%)	1 (1.2%)	1 (1.2%)	2 (2.5%)
Abdominal aorta (n=81)	2.19 (0.49) 2.21 [1.05-3.46]	2 (2.5%)	1 (1.2%)	3 (3.7%)	0 (0%)	2 (2.5%)	2 (2.5%)

n=number of values, SUV_{max}=maximum Standardised Uptake Value.

A total of 1125 ROIs in 12 tissues (and 2 background areas) were examined for the 81 cases. The distribution of SUV_{max} values in the tissues is shown in figure 1A. Lowest mean values were observed in hips, shoulders and salivary glands as compared to thyroid and aortic wall. Thirty-six values, belonging to 18 patients, exceeded the mean of SUV_{max} +2SD and 12 values, belonging to 5 patients exceeded the mean +3SD (4 hips, 2 shoulders, 2 parotids, 2 submandibular glands, 1 aortic arch and 1 thoracic aorta).

When we analyzed data of $SUV_{target}/SUV_{gluteus}$ ratios (Figure 1B), we found 33 values, belonging to 21 patients, exceeding the mean +2SD and 10 values, belonging to 7 patients exceeding the mean +3SD (2 hips, 1 shoulders, 2 submandibular glands, 2 thyroid, 1 thoracic aorta and 2 abdominal aorta).

Similarly, data of SUV_{target}/SUV_{aorta} ratios, showed 36 values, belonging to 23 patients, exceeding the mean +2SD and 13 values, belonging to 8 patients exceeding the mean +3SD (3 hips, 2 shoulders, 1 parotids, 1 submandibular glands, 1 thyroid, 1 aortic arch, 2 thoracic aorta and 2 abdominal aorta), as shown in figure 1C.

Figure 1

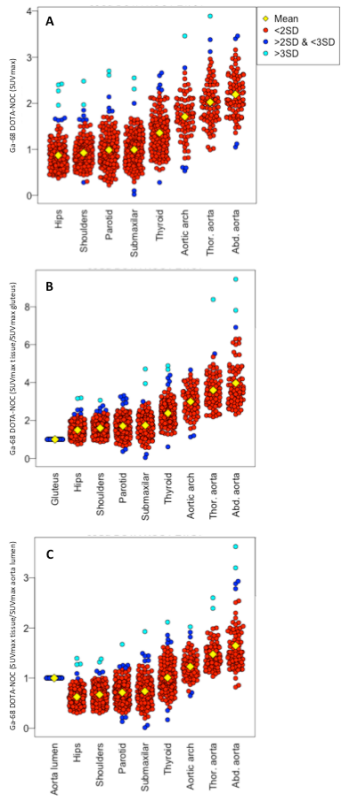

Figure 1. **Distribution of ^{68}Ga-DOTA-NOC in tissues.** A) SUV_{max} values in all the examined tissues. For hips, shoulders, parotid glands, submandibular glands and thyroid two measurements (left and right) were performed for each patient. B) Distribution of ratio SUV_{max} in tissue/SUV_{max} in gluteus muscle. C) Distribution of ratio SUV_{max} in tissue/SUV_{max} in aortic lumen. Yellow diamonds represent the mean. Dark blue and cyan circles represent the mean ±2SD and ±3SD respectively.

Of the 18 patients with SUV_{max} above the mean +2SD, only 5 had multiples sites of increased uptake (figure 2). Fifteen of these 18 patients accepted to have a biochemical and clinical screening for chronic inflammatory diseases (including rheumatology, immunology and endocrinology test). Only three subjects showed signs but no symptoms suggestive for arthritis or xerostomia (1 with SUV_{max} >3SD in joints; 1 with SUV_{max} >3SD in salivary glands; 1 with SUVmax >3SD in both joints, and salivary glands) but laboratory tests were negative in all of them. (Figures 3,4) Linear regression analyses showed that age and SUV_{max} were positively significantly correlated (corr. coeff. 0.001; $p<0.01$) in all tissues except abdominal aorta. SUV_{max}

mean in the tissues where not significantly correlated with sex, SST treatment (that may compete with DOTA-NOC) or scan positivity (reflecting underlying NET activity). The Spearman value between the three methods for analysing all tissues showed values greater than 0.85 between the global SUV_{max} method and the $SUV_{target}/SUV_{gluteus}$ for all tissues but aorta.

Discussion

In the present study we quantified the uptake of ^{68}Ga-DOTA-NOC in tissues that could be affected by chronic inflammatory process. To our knowledge this is the first report that analyses these tissues by SRS in normal subjects.

Patients were recruited based on the absence of any specific symptom and absence of any clinically diagnosed disease of salivary glands, thyroid, joints and large vessels. All patients with reported xerophtalmia, xerostomia, thyroiditis, hypo or hyperthyroidism, arthritis or any kind of vasculitis were excluded from this study. Nevertheless, we did not measure autoantibody titres in all patients, but only in 15 of the 18 patients who showed a high ^{68}Ga-DOTA-NOC uptake in some of the target tissues. This may be a limitation of our study but being a retrospective study, we had no possibility to access to all patients but only anamnestic data were available. Therefore, we cannot exclude that some of the patients with a low tissue uptake might have some autoantibody and an underlying autoimmune phenomenon. On the other hand, we did not aim at evaluating the sensitivity and specificity of ^{68}Ga-DOTA-NOC scan for autoimmune/inflammatory conditions in normal subjects, but rather we aimed at describing the range of uptake in tissues in a population without clinical signs or symptoms of autoimmune/inflammatory conditions.

The highest uptake value was found in the abdominal aorta followed by the thyroid gland, possibly explained by the presence of activated macrophages in atherosclerotic plaque as suggested by some authors (17) and the presence of lymphocytes with somatostatin receptors in thyroid gland (18). These values were not different among patients with or without a positive SRS for NETs, suggesting that the normal uptake pattern, in the examined tissues, is unaffected by the presence of tumour activity.

To quantify the radiopharmaceutical uptake in each tissue we used three different methods: SUV_{max}; $SUV_{target}/SUV_{gluteus}$ ratio as a subtraction of background activity; $SUV_{target}/SUV_{aorta\text{-}lumen}$ ratio as a subtraction of blood pool activity. We found a good correlation (Spearman values >0.85) between the three methods in all tissues, but in aortic segments. Therefore we could propose to use the global SUV_{max} method as an easy, simple and reproducible way to quantify the radiopharmaceutical uptake in all tissues but aortic wall, in which the measurement of $SUV_{target}/SUV_{aorta\text{-}lumen}$ ratio is more appropriate to subtract circulating activity.

Out of 81 patients, we found 5 patients with multiple sites of increased uptake and 3 of them had signs, but not symptoms, of impaired salivary gland function or arthrosis, despite the absence of specific auto-antibodies.

We cannot conclude that SRS can detect early inflammatory diseases by this study and an appropriate study should be designed to evaluate the role of SRS in these chronic disorders. In this context, our results are of relevance because they provide a standard reference of SST uptake in normal tissues with possible involvement of chronic inflammatory disorders, to be used to identify patients with pathological uptake.

As we know autoimmune or chronic inflammatory diseases are characterized by phases of exacerbation and quiescence. Clinical and biochemical findings may be present or not and the use of SRS could help to objectively identify patients with a pathological lympho monocitic infiltration in tissues (19).

Prasad et al. (14) in 2010 reported higher SUV_{max} values, than ours, of ^{68}Ga-DOTA-NOC in thyroid (4.7±2.2 vs 1.36±0.45) and in parotid glands (1.9±0.6 vs 0.98±0.4). Also Kuyumcu et al.(20), using ^{68}Ga-DOTA-TATE, found a SUV_{max} in normal thyroid of 4.18±1.9. However, neither Prasad or Kuyumcu tested their patients for auto-antibodies and we cannot exclude the presence of patients with thyroiditis or Sjögren's syndrome amongst them, that have raised the level of SUV_{max} in glands.

The main limitation of our study is the retrospective analysis of a population with oncologic disease, some under treatment with SST analogues. However, we did not find any significant difference between patients with and without an SRS positive tumor or between patients treated and non-treated with SST analogues. The number

of examined patients may appear too exiguous to carry out definitive conclusions although the small standard deviation values found for each tissue, encourage to sustain the correctness of the sample size.

This study is the first that examine the biodistribution of ^{68}Ga-DOTA-NOC in normal salivary glands, thyroid, major joints and aortic wall, thus providing as future research directions a reference for further studies in patients affected by chronic inflammatory disorders or with a suspicion of it.

Figure 2

Figure 2. An example of a patient with uptake levels in thyroid gland higher than population mean +2SD. This patient had no signs or symptoms of thyroid dysfunction neither the presence of autoantibodies.

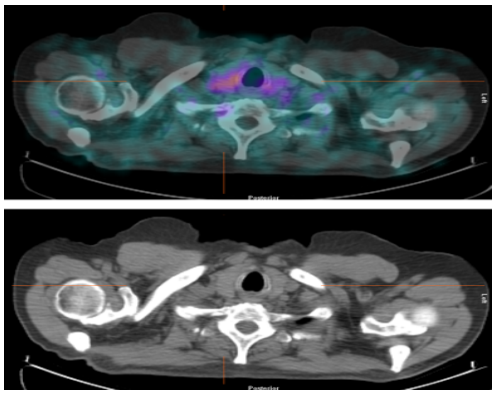

Figure 2. An example of a patient with uptake levels in thyroid gland higher than population mean +2SD. This patient had no signs or symptoms of thyroid dysfunction neither the presence of autoantibodies.

Figure 3

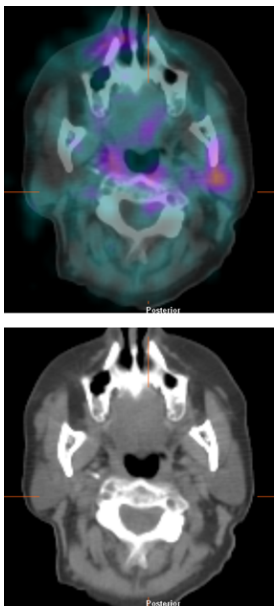

Figure 3. An example of a patient with uptake levels in left parotid gland higher than population mean +3SD. This patient had signs of xerostomia but no symptoms nor autoantibodies. The rheumatologist asked for a salivary gland scintigraphy that showed mild impairment of parotid function.

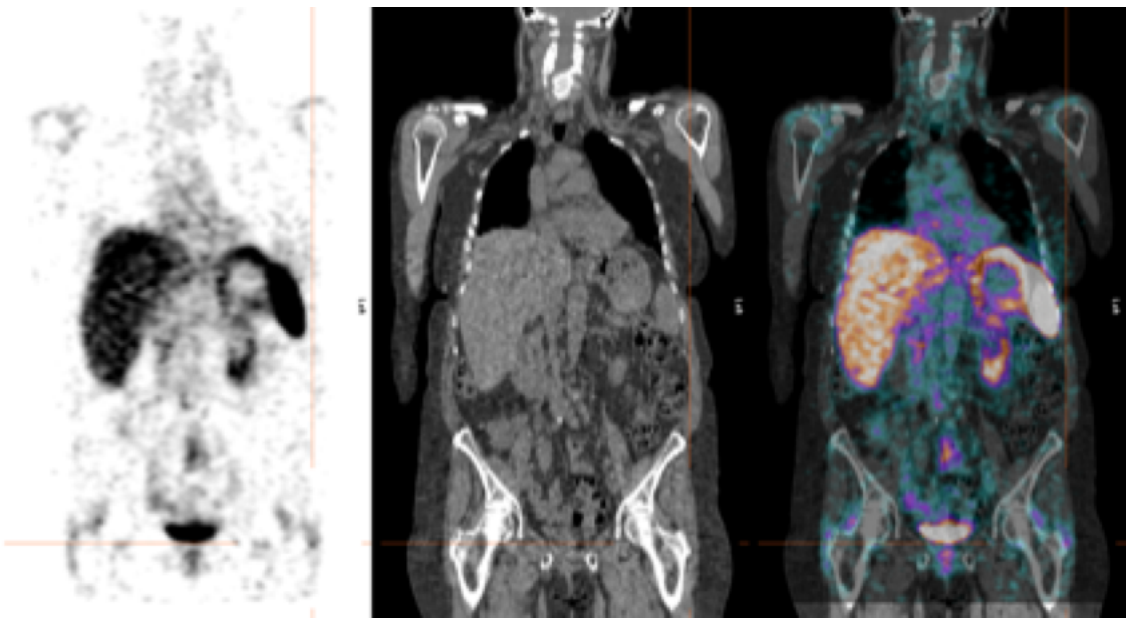

Figure 4. An example of a patient with uptake levels in shoulders and hips higher than population mean +2SD. This patient had inducible pain in joints but no symptoms nor autoantibodies. He was diagnosed with initial arthropathy.

Conclusions

We quantified the uptake of ^{68}Ga-DOTA-NOC in different tissues that might be affected by a chronic inflammation. These values could be used to identify the cut-off to discriminate between normal or pathological condition in a ^{68}Ga-DOTA-NOC PET/CT and to monitor the course of autoimmune or chronic inflammatory diseases.

Conflicts of interest

The authors certify that there is no conflict of onterest with any financial organization regarding the material discussed in the manuscript.

Authors' contribution

All authors equally contributed: Formal analysis, Luz Kelly Anzola and Alberto Signore; Investigation, Carlos Granados and Bruno Lagana; Resources, Chiara Lauri; Supervision, Alberto Signore; Writing – original draft, Luz Kelly Anzola.

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Chapter 7
Summary in English

Nuclear medicine techniques for imaging inflammation have had huge progress and have enormously expanded over the past 20 years in parallel with the understanding of the pathogenesis of chronic inflammatory diseases and the role of different immune cells and cytokines. Several new radiopharmaceuticals have been developed, able to detect early and late pathologic events at a molecular level. The name “molecular nuclear medicine”, therefore, refers to all those radiopharmaceuticals and techniques used in nuclear medicine to visualize, and often quantify, the molecular events involved in a disease. We cannot stress enough the importance of this branch of medicine not only in the diagnostic setting but also in prognosis and treatment management. The key clinical feature of molecular nuclear medicine relies on the ability to detect in-vivo the components and phases of diverse disorders such as neoplastic diseases and inflammatory conditions, besides the solely morphological changes often detected by most other imaging procedures. This characteristic provides the rationale for the use of molecular imaging techniques for early diagnosis and treatment decision making.

In this monography, we have focused mainly on the contribution and the role of molecular nuclear medicine to detect early phases of chronic inflammation by the use of radiolabelled peptides and monoclonal antibodies (mAbs).

Several radiopharmaceuticals have been used for imaging different chronic inflammatory conditions. Their mechanisms of accumulation in chronic lymphocytic inflammatory sites varies; for non-specific radiopharmaceuticals such as ^{67}Ga-citrate and ^{99m}Tc-HIG, the accumulation in tissues depends upon enhanced capillary transudation, secondary to increased vascular permeability and increased blood supply. Tissue accumulation of more specific radiopharmaceuticals such as ^{99m}TcmAbs and ^{99m}Tc-cytokines depends upon the antigen-antibody interaction or a specific receptor-binding process, thus allowing the histopathological characterization of the inflammatory process and the definition of its severity and

type. Even though there is not one single ideal radiopharmaceutical for imaging all chronic inflammatory diseases, some combination of them could be used for the complete understanding of the histopathology involved and, therefore, to identify a specific and tailored therapy. Moreover, these novel tools can detect cell binding and the presence of cytokines in patients suspected of an inflammatory condition based on laboratory tests. These agents can also demonstrate active inflammation in patients without systemic inflammatory response and can predict response to treatment. Today, clinicians have the possibility to choose between different options according to the purpose and clinical requirements.

Radiolabelled cytokines and mAbs are an emerging class of molecules for imaging inflammation. These radiopharmaceuticals bind to their targets with high affinity and specificity, and therefore have excellent diagnostic potential for imaging patients with chronic inflammatory diseases.

One of the key type of cytokines involved in the process of inflammation is tumor necrosis factor alpha (TNFα). With the introduction of anti-TNFα monoclonal antibodies over the past decade, treatment of inflammatory diseaseshas evolved, with remarkable contributions in controlling signs and symptoms of inflammation and slowing tissue destruction. However, some of these drugs may lose efficacy over time or induce adverse events in many patients.Prompt application to the right medication tailored to the patient's molecular status avoids unnecessary costs; labelled agents may help to find out whether TNFα is present in the inflammatory process and could, as a result, help in prediction of therapeutic efficacy and stratification in each specific patient.

Currently available evidence shows different possibilities of labelled anti-TNFα antibodies like ^{99m}Tc-Infliximab and ^{99m}Tc-Adalimumab for diagnosis and therapy, with great potential in clinical practice. Published reports have demonstrated that molecular imaging with anti-TNFα MoAbs can be used for cost-effective treatment decisions such as selection of patients who are best candidates for anti-TNFα therapy.

Somatostatin is a cyclic hormone that regulates several cell processes via specific receptors expressed throughout the body. This hormone was initially known only

as a regulating factor but today is well recognized as a potent drug and imaging tool. The discovery of its different receptors and molecular subtypes gave raise to interdisciplinary research, leading to the use of somatostatin analogues in routine clinical practice. The high density expression of these receptors in different inflammatory cells and tissues, has allowed chronic inflammatory diseases such as RA, Sjrogen disease, idiophatic pulmonary fibrosis, amongst others, to be evaluated with different radiolabelled analogue somatostatin petides to be used with SPECT and PET with encouraging results, showing interesting clinical potential. Reported evidence is mainly supported by observational studies designed to investigate different groups of chronic inflammatory conditions:

For endothelial inflammation and vulnerable plaques, the most frequent pathology evaluated with PET molecules (^{68}GaDOTA NOC-TEC-TOC), promising correlations were described between quantitative uptake and histopathology, emphasizing the role of SRS for this condition.

In idiopathic pulmonary fibrosis, the reported experience shows attractive results, emphasizing the utility of ^{68}Ga and ^{111}In tracers with high concordance with other imaging and functional techniques, demonstrating the activity degree of inflammatory processes with implications in prognosis and therapeutic decision making.

RA and Sjögren's disease were also positively impacted by the radioabelled somatostatine analogues. Today's evidence shows the capacity of this tool to identify sites of active inflammatory and to predict which joints could be successfully treated with biologic drugs. In Sjögren's disease, these novel molecular imaging tools for the first time permit the evaluation of the whole body and allows the detection of secondary sites of non-suspected active inflammation. that traditionally were not possible to evaluate with conventional techniques. Furthermore, these radiolabelled agents showed significant superiority to identify salivary gland compromise compared to sialoscintigraphy, currently included in the ACR/EULAR criteria.

The overall evidence shows that SRS is able to play a crucial role for diagnosis, prognosis and therapy response in different chronic inflammatory diseases. This

monography is intended to enrich the vast field of nuclear medicine by gathering in a comprehensive manner the current experience of theranostics in inflammatory diseases. As researches and investigators, it becomes necessary to invite all those interested in this exciting territory to explore the breakthroughs emerged during the 5 years this investigation lasted.

Chapter 8
Samenvatting in het Nederlands

Nucleair geneeskundige technieken om ontstekingen in beeld te brengen hebben enorme vooruitgang geboekt en zijn de afgelopen 20 jaar enorm uitgebreid, parallel met het begrip van de pathogenese van chronische ontstekingsziekten en de rol van verschillende immuuncellen en cytokines.

Verschillende nieuwe radiofarmaca zijn ontwikkeld om vroege en late gebeurtenissen op moleculair niveau in beeld te brengen. De naam "moleculaire nucleaire geneeskunde" verwijst daarom naar al die radiofarmaca en technieken die in de nucleaire geneeskunde worden gebruikt om het molecuul en de moleculaire gebeurtenissen die bij een ziekte betrokken zijn, te visualiseren en vaak te kwantificeren. We kunnen niet benadrukken hoe belangrijk deze techniek is in zowel de diagnostische setting als voor prognostische implicaties en management van de behandeling. De klinische rol van moleculaire nucleaire geneeskunde is afhankelijk van het vermogen van functionele beeldvorming om, in vivo, de componenten en fasen van verschillende ziekten, zoals tumoren en ontstekingsziekten, te detecteren, naast de pure morfologische afwijkingen die worden afgebeeld met radiologische procedures. Dit kenmerk is de reden voor het gebruik van de nucleaire geneeskunde technieken voor vroege diagnose en besluitvorming over de behandeling.

In dit proefschrift hebben we ons vooral gericht op de bijdrage en de rol van moleculaire nucleaire geneeskunde om vroege fasen van chronische ontstekingsziekten te detecteren door het gebruik van radioactief gemerkte peptiden en monoclonale antilichamen (mAbs).

Verschillende radiofarmaca zijn gebruikt voor scintigrafische beeldvorming van verschillende chronische ontstekingsziekten. Hun accumulatiemechanisme op chronische lymfocytaire ontstekingsplaatsen varieert; voor niet-specifieke radiofarmaca (zoals ^{67}Ga-citraat en ^{99m}Tc-HIG) hangt de accumulatie in weefsels

af van een toegenomen capillaire transudatie, secundair aan verhoogde vasculaire permeabiliteit en verhoogde bloedtoevoer.

Weefselaccumulatie van meer specifieke radiofarmaca (zoals ^{99m}Tcm-Abs, ^{99m}Tc-cytokines) hangt af van de antigeen-antilichaaminteractie of van een specifiek receptorbindingsproces, waardoor de histopathologische karakterisering van het ontstekingsproces en de definitie van de ernst en het type ervan mogelijk wordt. Hoewel er niet één ideaal radiofarmaceutisch middel is voor het in beeld brengen van alle chronische ontstekingsziekten, kan een combinatie van meerdere tracers worden gebruikt voor een volledig begrip van de histopathologie en daardoor om een specifieke en op maat gemaakte remedie te identificeren. Bovendien kunnen die nieuwe hulpmiddelen de aanwezigheid van cytokines in ontstekingsplaatsen detecteren bij patiënten die op basis van laboratoriumtests worden verdacht van een ontstekingsziekte.

Het kan ook actieve ontsteking aantonen bij patiënten zonder systemische ontstekingsreactie en kan de respons op de behandeling voorspellen.

Tegenwoordig hebben artsen de mogelijkheid om te kiezen tussen verschillende opties, afhankelijk van het doel en de klinische implicaties.

Radioactief gelabelde cytokines en monoklonale antilichamen zijn een opkomende klasse van radiofarmaca voor beeldvorming. Deze radiofarmaca binden zich met hoge affiniteit en specificiteit aan hun doelen en hebben hierdoor een uitstekend diagnostisch potentieel voor beeldvorming van patiënten met chronische ontstekingsziekten.

Een van de belangrijkste cytokines die betrokken zijn bij het proces van ontsteking is tumor necrose factor alfa (TNFα). Met de introductie van anti-TNFa-monoklonale antilichamen in het afgelopen decennium is de behandeling van ontstekingsziekten geëvolueerd. Verder heeft het ook opmerkelijke voordelen mogelijk gemaakt bij het beheersen van symptomen van ontsteking en bij het vertragen van destructie. Geneesmiddelen kunnen na verloop van tijd echter hun efficiëntie verliezen bij patiënten of bijwerkingen veroorzaken. Door onmiddellijk de juiste medicatie te gebruiken die is afgestemd op de moleculaire status van de patiënt, worden onnodige kosten voorkomen. Het

labelen van deze middelen kan helpen om uit te vinden of TNFa aanwezig is in het ontstekingsproces en zal, als resultaat, helpen bij de therapie voorspelling en stratificatie bij elke specifieke patiënt.

Het gepubliceerde bewijs tot vandaag toont verschillende scintigrafische mogelijkheden van gelabelde anti-TNFα-antilichamen voor diagnose en therapie-evaluatie: ^{99m}Tc-Infliximab, en ^{99m}Tc-Adalimumab met een groot potentieel in de klinische praktijk. Gepubliceerde rapporten hebben zonder twijfel aangetoond dat moleculaire beeldvorming via anti-TNFα mAbs kan worden gebruikt voor behandelbeslissingen en selectie van patiënten die in aanmerking komen voor anti-TNFα-therapie. Deze benadering kan een cruciale rol spelen bij het selecteren van de beste therapeutische optie voor elke patiënt en het vermijden van extra kosten door ineffectieve biologische therapieën.

Somatostatine is een cyclisch hormoon dat verschillende fysiologische celprocessen reguleert via specifieke receptoren die door het hele lichaam tot expressie worden gebracht. Dit hormoon stond in het begin alleen bekend als een regulerende factor en is tegenwoordig bekend als een goed medicamenteus middel en is geschikt voor beeldvorming. De ontdekking van de verschillende receptoren en subtypen gaf aanleiding tot interdisciplinair onderzoek dat leidde tot het gebruik van somatostatine-analogen in de routine klinische praktijk. De hoge dichtheid expressie van deze receptoren in verschillende ontstekingscellen en weefsels, heeft ertoe geleid dat chronische ontstekingsziekten zoals RA, de ziekte van Sjögren, en idiopatische longfibrose, kunnen worden geëvalueerd met verschillende radioactief gelabelde analoge somatostatinereceptoren; voor diagnostische doeleinden zijn deze receptoren radioactief gelabeld voor SPECT- en PET- beeldvorming met bemoedigende resultaten, met een interessant potentieel in de dagelijkse klinische praktijk. Het tot nu toe gepubliceerde bewijs wordt voornamelijk ondersteund door observationele studies die zijn ontworpen om verschillende groepen chronische ontstekingsziektente onderzoeken.

Voor inflammatie van endotheelcellen en vulnerabele plaques, de meest voorkomende ziekten die geëvalueerd zijn met PET- tracers (^{68}Ga-DOTA-NOC-TEC-TOC), werden veelbelovende correlaties beschreven tussen kwantitatieve

opname en histopathologie, waarbij de nadruk werd gelegd op de rol van SRS voor deze inflammatoire aandoening.

Voor idiopathische longfibrose zijn ook aantrekkelijke resultaten zichtbaar, met nadruk op het nut van ^{68}Ga en ^{111}In tracers die een hoge correlatie lieten zien met andere beeldvormende technieken; het benadrukte ook de activiteit van ontstekingsprocessen en de implicaties als prognostische factor en wordt gebruikt in een context van besluitvorming.

Rheumatoide arthritis en de ziekte van Sjögren worden ook positief beïnvloed door de radioactief gemerkte somatostatine-receptoren. Het bewijs tot vandaag laat zien dat deze beeldvormende techniek in staat is om locaties van actieve ontstekingsprocessen te identificeren en te voorspellen welke gewrichten met succes kunnen worden behandeld met biologische geneesmiddelen. Verrassend genoeg toont deze techniek bij de ziekte van Sjögren voor het eerst de mogelijkheid om het hele lichaam te evalueren en verschillende plaatsen van actieve ontstekingsprocessen secundair aan de ziekte te detecteren die niet werden vermoed, en dat traditioneel niet mogelijk was met andere technieken. Ongetwijfeld zijn deze radioactief gemerkte receptoren ook superieur om inflammatie van de speekselklieren te identificeren ten opzichte van de sialoscintigrafie, tot vandaag geaccepteerd in de EULAR-criteria.

Sommige studies van speciaal belang evalueerden de theranostische rol van SRS om patiënten te identificeren die baat zouden kunnen hebben bij behandeling met somatostatine-analogen en / of biologische geneesmiddelen die een goede respons op therapie garanderen. Het algemene bewijs toont aan dat SRS een cruciale rol kan spelen bij de follow-up van ziekte, de diagnose, de prognose en de therapiekeuze bij verschillende chronische ontstekingsziekten. In het licht van de enorme omvang van het nucleaire geneeskundige arsenaal, verrijkt de bijdrage in dit proefschrift de eerder verrichte studies. Verschillende nieuwe ontwikkelingen en bevestiging van bestande methoden werden gevonden in verschillende inflammatoire laesies. Als onderzoeker is het noodzakelijk om al diegenen die geïnteresseerd zijn in dit

overweldigende gebied uit te nodigen om de doorbraken te verkennen die zijn verworven in de 5 jaar dat dit onderzoek duurde.

Chapter 9

Conclusions and future perspectives

The concept of molecular imaging that defines nuclear medicine practice is clearly and well appreciated in the clinical approach of chronic inflammatory diseases where novel molecules have been developed for diagnostic and prognostic purposes, with a clear potential cost/effective role. It is fascinating how radiolabelled somatostatin receptors are used today in different scenarios other than neoplastic diseases; the medical evidence presented in this review highlights the use of these tools in other diseases such as Graves' ophthalmopathy, rheumatoid arthritis, granulomatous diseases, idiopathic pulmonary fibrosis, amongst other inflammatory conditions, allowing to evaluate the response to specific therapeutic approaches and predicting who might benefit from them. In a more sophisticated level, it is important to remark the possibility to radiolabel biological compounds through the use of monoclonal antibodies, as in the case of anti TNF-alfa MoAb, which have shown to facilitate a customized clinical management adapted to the requirements of each patient, helping to select the best candidates for biologic therapies and assuring success, therefore improving the quality of life, representing with a favourable cost/benefit ratio for our health systems.

The promising results published to date about the usefulness of radiolabelled somatostatine analogs and some monoclonal antibodies such as anti TNF-alfa MoAb in the field of chronic inflammatory diseases, should encourage the scientific community to move forward to conduct novel and better designed harmonized research protocols, with larger and more homogenous patient groups in order to achieve robust results, making these techniques part of the daily practice in different settings. It is evident how significant is to validate these techniques using PET and SPECT technology, to improve the quantification methods, to standardize imaging interpretation and to determine the clinical usefulness of specific tracers for both theranostics and follow-up. We believe that somatostatine analogues could be radiolabelled with beta- or alfa-emitters for treatment purposes, as in the case of refractory rheumatoid arthritis, Graves' orbitopathy, and bringing novelty in cases

such as the vulnerable atheromatous plaque. Considering the poor prognosis of some clinical conditions, as well as the limited effectiveness and high prices of some current treatments, our observations may lead to the delineation of new therapeutic approaches using radiolabelled somatostatin analogues and monoclonal antibodies. Completing this review and provided, as considered by myself, one of the most comprehensive written evidence in the field of chronic inflammatory diseases, has been an effortful job. Nothing would make me feel more overjoyed, to both invite and encourage you to continue researching, investigating, and realizing new accomplishments using this thesis material as a primary bedrock. As scientists, we must elevate any knowledge acquired with the simplest but worthiest repercussion in our society, this is the comprehension of the diseases exposed in order to help people to have a better life.

Chapter 10

CURRICULUM VITAE – LUZ KELLY ANZOLA FUENTES

LUZ KELLY ANZOLA FUENTES, MD
Date of birth: 16TH SEPTEMBER 1963
Place of birth: CALI, COLOMBIA

AFFILIATION

Nuclear medicine unit in diagnostic imaging department, Clinicas Colsanitas, Bogota, Colombia.
Nuclear medicine postgraduate program in medicine faculty, Fundacion Universitaria Sanitas, Bogota, Colombia.

EDUCATION

1992 Specialization in Nuclear Medicine Universidad Javeriana, Bogota Colombia.
1987 University degree in Medicine Universidad Javeriana, Bogota Colombia
2016 Fellow in Epidemiology and Clinical Trials Harvard Medical School, Boston.

PRESENT ACTIVITY

National director of nuclear medicine for Clinicas Colsanitas in Colombia.
Director of the nuclear medicine postgraduate programme in medicine faculty at Fundacion Universitaria Sanitas, Bogota, Colombia.
Active member of the Colombian Society of Nuclear Medicine.
Associate member of the Colombian Society of Orthopaedics.
Active member of the American Society of Nuclear Medicine.
Active member of the International Research Group in Immuno-Scintigraphy and Therapy-IRIST group.
Member for the International board for IRIST group.
President of IRIST group for the period 2016-2018.

Associate professor in nuclear medicine postgraduate programme at Fundacion Universitaria Sanitas, Bogota, Colombia,

SCIENTIFIC ACTIVITY

Author of scientific publications in national and international journals and book chapters. Editor and author of 2 books.

LANGUAGES

Spanish speaking mother tongue.
Good spoken and written English. B2
Good spoken and written Italian. B2

PROFESSIONAL EXPERIENCE

1993-present Chief Nuclear Medicine Department Clinicas Colsanitas, Bogota, Colombia.
2009-present Director of the nuclear medicine postgraduate program at Fundacion Universitaria Sanitas, Bogota, Colombia.
1999 Short clinical training in Sport Injuries and nuclear medicine,Maryland University, USA.
1996 Short Clinical Training in neuroimaging with SPECT. Illinois University, USA.

NATIONAL AWARDS AND HONORS

2014 President of 22nd IRIST International meeting, Cancun, Mexico.
2016 President of the International Research Group in Immunoscintigraphy and therapy-IRIST group.

PUBLICATIONS

1.G. Malviya, K. **L. Anzola**,E. Podestà, B. Laganà, C. Del Mastro, R. A. Dierckx, F. Scopinaro, A. Signore. Tc-labeled Rituximab for Imaging B Lymphocyte Infiltration in Inflammatory Autoimmune Disease Patients. Mol Imaging Biol .(2012) 14:637Y646. DOI: 10.1007/s11307-011-0527-x.

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Chapter 11

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Chapter 12.

STELLINGEN

1. The use of radiolabelled antibodies for diagnostic purposes allows evidence-based biological therapy with the same, unlabelled antibody. (This thesis, chapter 1).

2. ^{68}Ga-DOTA-TOC can be used to detect high risk, vulnerable, atherosclerotic plaques. (This thesis, chapter 2).

3. Somatostatin receptor scintigraphy with ^{68}Ga-DOTA-TOC has clinical value in many chronic inflammatory disorders such as Graves' ophthalmopathy, pulmonary fibrosis and Rheumatoid Arthritis. (This thesis, chapter 2).

4. Scintigraphy with ^{99m}Tc-rituximab demonstrates the presence of B lymphocyte infiltration in affected joints of patients with arthritis, thus providing evidence for treating these lesions with unlabelled Rituximab. (This thesis, chapter 3).

5. Somatostatin receptor scintigraphy can assess disease activity in Rheumatoid Arthritis patients and detects salivary gland inflammation in patients with secondary Sjögren's syndrome. (This thesis, chapter 4).

6. Somatostatin receptor scintigraphy as whole body imaging technique is able to identify the involvement in salivary glands as well in the joints and other tissues in patients with primary Sjögren's Syndrome. (This thesis, chapter 5).

7. ^{68}Ga-DOTA-NOC PET/CT can also be used instead of ^{99m}Tc-HYNIC-TOC scintigraphy for imaging tissues affected by chronic inflammation. It is, therefore, important to define the cut-off values of "normality" in order to diagnose and monitor chronic inflammatory diseases. (This thesis, chapter 6).

8. The real voyage of discovery consists not in seeking new lands, but seeing with new eyes. (Marcel Proust).

9. The perfect blend for having no limits is having the curiosity of your inner child, the thrive of your puberty and the wisdom of your adulthood. (Kelly Anzola).

10. The higher the dream the wealthier the journey. (Kelly Anzola).

11. When I look back at the years of struggle, I find them to be the most beautiful and useful to build up my future. (Kelly Anzola).